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The Inapplicability of the Subspecies Concept

In North American Marten — *E. M. Hagmeier* — 1

In a Collembolan (Hexapoda) — *K. Christiansen* — 8

On the Origin of the Eumetazoa — *E. D. Hanson* — 16

Points of View

Characters and Variation in Mollusks • *Burch & Thompson* — 48

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Inapplicability of the Subspecies Concept to North American Marten

EDWIN M. HAGMEIER

ORTHODOX systematic treatment of North American martens (*Martes americana*) led me to conclude that partitioning of the species into subspecies was completely arbitrary, because most variation was clinial, and because at least some characters varied discordantly. An attempt was made to test this conclusion by mapping characters singly as two-dimensional clines, and in combination as averages of slopes of clines in the manner suggested by Womble (1951). This paper is devoted to an account of the testing and its results.

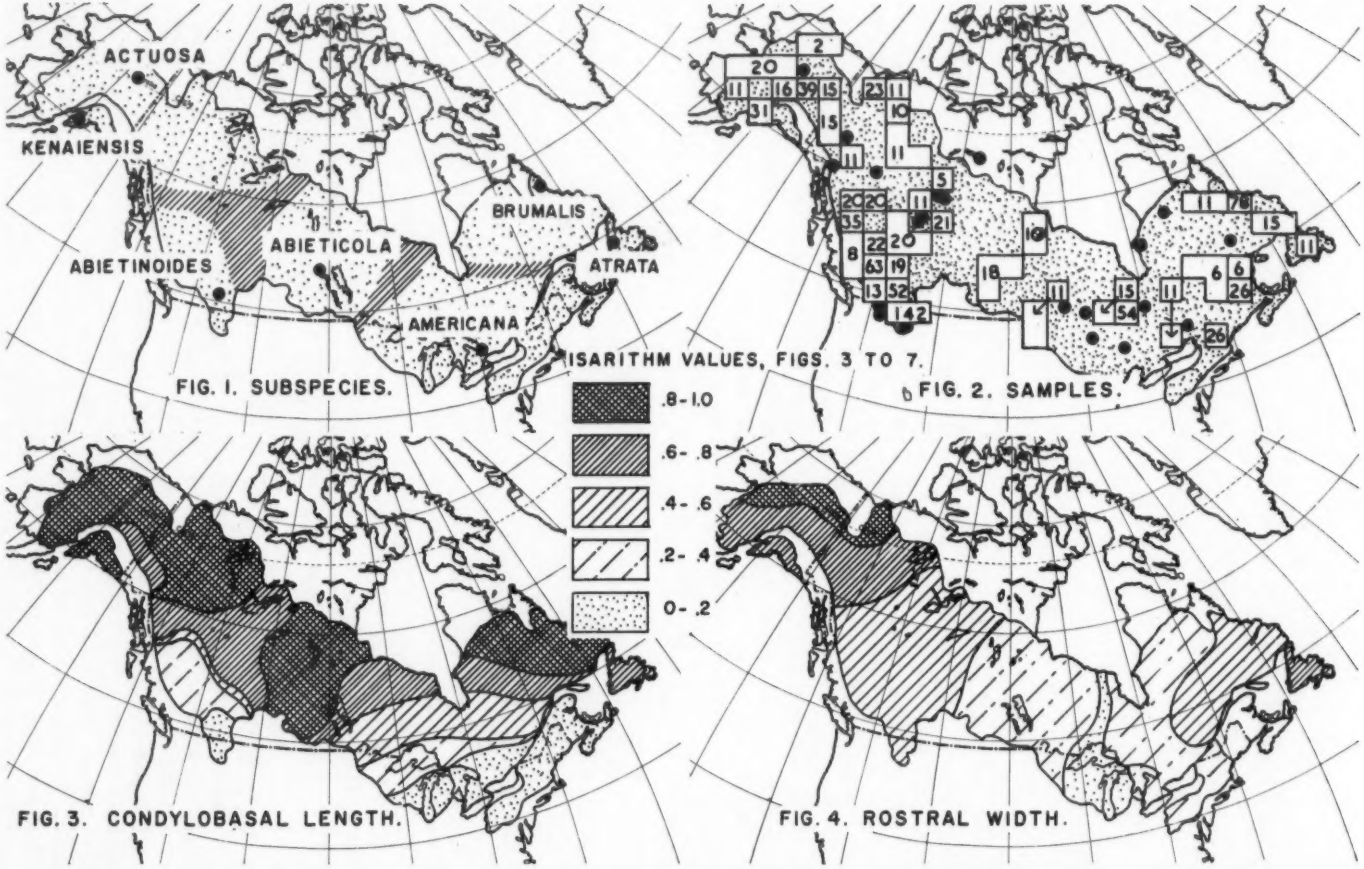
American marten all belong to a single species, *M. americana* (Wright, 1953), which is closely related to, and may be conspecific with, *M. zibellina* and *M. martes* of Eurasia (Hagmeier, 1955). Wright (op. cit.) has concluded that *M. americana* is made up of two morphological "sections," *americana* and *caurina*, which intergrade where their ranges meet in Idaho and Montana. The work reported here is concerned only with variation in the *americana* section of *Martes americana*. This section is currently described as comprising seven subspecies, whose ranges and type localities are mapped as Figure 1.

Method

The skulls of 1803 individuals referable to *M. americana* section *americana* were examined. Specimens were sexed and aged according to methods outlined elsewhere (Hagmeier, 1955). Twenty-four measurements were first taken on a small (n=21) sample from southeast British Columbia. Twelve characters with relatively low coefficients of variability were selected from these, and measured on 1044 of the specimens, most of which had been classified as "mature." The measurements were: (1) condylobasal length; (2) rostral width; (3) upper tooth row; (4) canine width at base; (5) bulla length; (6) upper molar width; (7) upper molar inner moiety length; (8) palate length; (9) mastoid width; (10) height of skull at bullae; (11) length of last upper premolar; (12) narrowest palate width.

Skull measurements of females were compared to those of males from the same locality. Comparable measurements for all twelve were found to be 1.10 times larger in males than in females. When the measurements of females were multiplied by 1.10, and compared to males, t was not significant at the .05 level. In order to increase sample size, all female measurements were multiplied by 1.10 and pooled with those of males. Subsamples were then grouped by subspecies and tested by analysis of variance. All characters were found to give a significant value of F at the .05 level of probability.

Locality subsample means were then mapped, and isarithms (isopleths) fitted. Comparison of the twelve maps resulting showed variation to be essentially similar in all characters. Size of marten, as measured by condylobasal length, displays marked geographic variation, and it was suspected that it affected all characters, and masked underlying variation. Correlation coefficients were obtained for each character relative to condylobasal length. The significance of these were tested by t, and this for all characters except 6 and 7 was found to be significant. All characters were then divided by condylobasal length, grouped by subspecies, and tested by analysis of variance. No significance



FIGS. 1–4. See text for explanation. Note that in Figure 2 the solid circles represent localities, the samples from which comprise fewer than five individuals.

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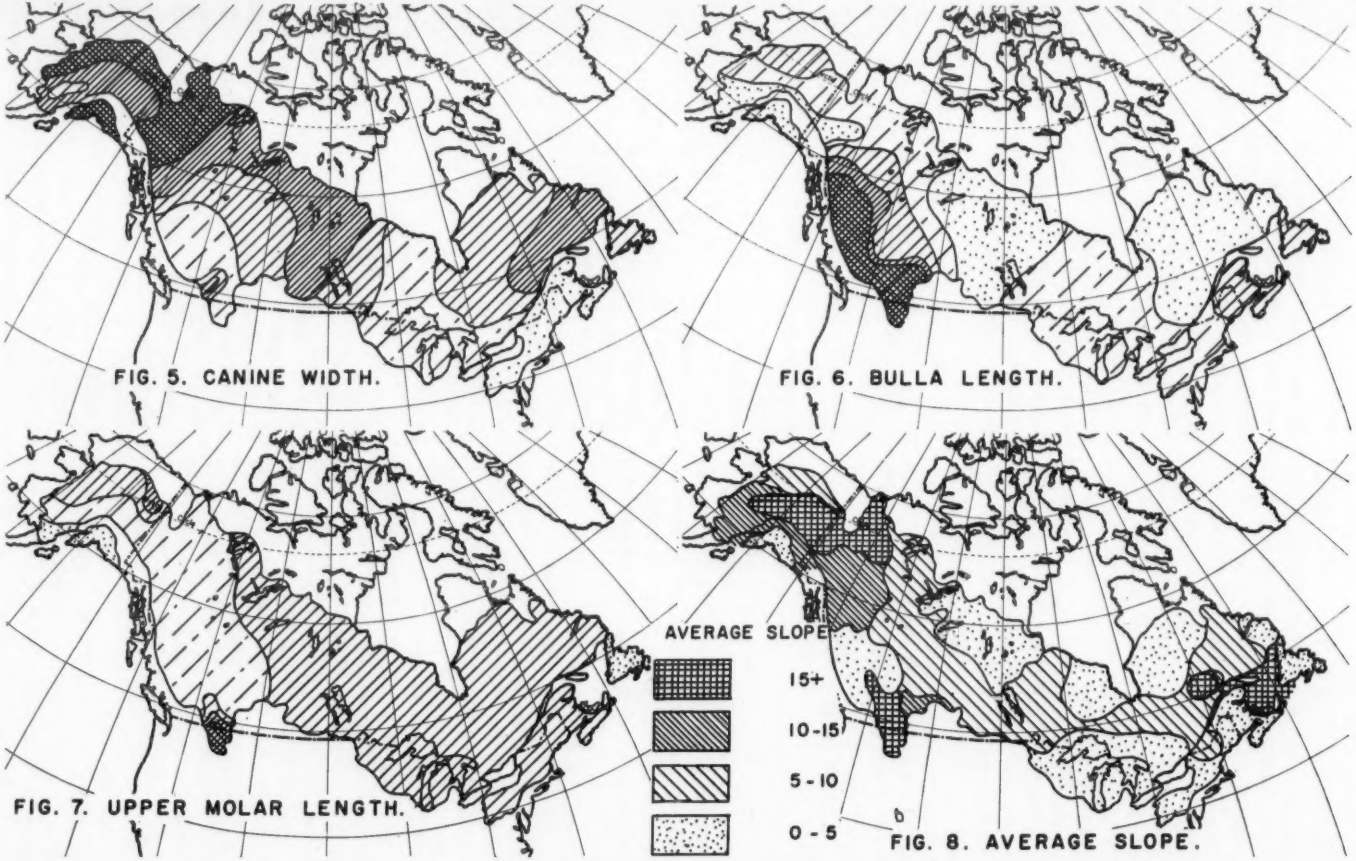

FIGS. 5–8. See text for explanation

(at the .05 level) was found in characters 3, 6, 8, 9, 10, 11 and 12. I concluded that these showed variation in size only, and that this was best illustrated by character 1 (condylobasal length). They were therefore discarded. Those remaining (except 1) were subsequently treated as the ratio of the character measured divided by condylobasal length.

In order that all characters would be weighted equally, the observed geographic range of each was fitted into the range 0 to 1.0. The values of both are given in Table I, so that these may be converted back to the original ratio (and in character 1 to the original measurement). With them are given an estimate of geographic variation expressed as a percentage of the mean of the whole of the *americana* section.

Because each local sample was small, a grid made up of blocks 100 miles to a side was laid at random over a map showing localities of samples. All samples within a block were pooled, thereby increasing sample size. The distribution of pooled blocks and sample sizes is shown on Figure 2.

The weighted and pooled sample means were plotted by block on maps of scale 100 miles to 1.25 inches, and isarithms (with a contour interval of 0.1) fitted. These maps on a smaller scale, simplified, and with a contour interval of .2 are shown as Figures 3, 4, 5, 6 and 7.

The fitting of isarithms is exceedingly subjective. Some control over sampling error was obtained by using Snedecor's equation (1948, p. 457), which showed that deviation of about ten per cent of total geographic variation on either side of the true mean was likely (at the .05 level) with samples of about less than eight variates. Samples (blocks) smaller than this were therefore treated as of doubtful value, and extreme means of these not mapped.

In order that the effects of geographic variation might be summarized on a single map, Womble's method (1951) was followed. For each isarithm map, slopes of isarithms were computed at randomly selected localities. The slopes at each locality were then multiplied (for convenience) by 10,000, and averaged. The averages were then mapped, and isarithms fitted. The map resulting is given as Figure 8. Its topography shows the rate of character change geographically.

The actual mechanics of computation and mapping were considerably more cumbersome and involved than I have outlined. I have, however, sketched the essential nature of the operations.

Discussion of the Methods

I believe that the approach to systematics taken here, while not new, has advantages over more orthodox methods. Its virtues lie in the fact that in an adequately refined condition, it is (or should be) repeatable, that knowledge obtained subsequently can be incorporated into it, and that it is objective to the extent that it attempts to describe variation before it partitions into subspecies. These qualities are often absent in systematic procedure at the infraspecific level.

On the other hand the approach is primitive, and gives only approximate results. There do not seem to be any adequate statistics available for the study of gradient phenomena distributed in two dimensions (Anderson, 1954). It was very difficult to control sampling error in the work reported here. In addition there is the fact that extensive areas of the section's range were not represented by any samples at all (Fig. 2). At least some apparent variation shown on the maps is due to error derived from these sources.

The small size of samples obtainable made it necessary to pool samples into blocks, and to convert and pool female measurements with those of males. Both procedures are probably justified on grounds of expediency. They do not invalidate the approach though they cast some doubt on the reliability of the findings in the present case.

Twenty mammalian systematic studies

made between the years 1909 and 1955, and selected at random, were examined so that what passes as acceptable sample size in studying variation could be determined. The average number of specimens examined per subspecies was 118.7 (40 to 270), and the average number of specimens examined per square mile of the species range (times 1,000) was .977 (.183 to 3.984). The comparable statistics for the study reported here (based on number of specimens measured) were 149 and .349. It appears from these that the marten specimens examined were fewer per unit area of range than in most systematic studies, but greater in number per subspecies. Perhaps marten have fewer named subspecies than other forms because there is less material available. Nevertheless the accepted subspecies of marten were named on the basis of the material available, and should be capable of re-examination through it.

The uneven distribution of samples (shown in Fig. 2) made it difficult to fit isarithms. Two individuals fitting contours to the same information may produce quite dissimilar maps (Mackay, 1951), and attempts have been made to control such differences of interpretation (Blumenstock, 1953; Krumbein, 1955; Mackay, 1955). None of these was exactly suited to the mapping done here, and I was obliged to use analysis of variance and Snedecor's test. They were helpful, but the maps are still approximations only.

In several well represented areas, especially in southern British Columbia, actual character gradients were evident. In most other cases I have had to assume that gradation occurs, and fit lines accordingly. This is probably a sound assumption, since character gradation has been found between the *caurina* and *americana* sections of the species. Nevertheless absolute proof of intergradation was often lacking.

The selection of taxonomic characters is always arbitrary. Of twenty-four measurements originally selected, only five were retained. Such selection is, as Gillham (1956) has pointed out, suppression of evidence. I believe that the removal of the first twelve characters (due to the relatively high coefficient of variability within samples) was justified in view of the relatively small inter-sample variation (see Table I). The subsequent removal of another seven, because they showed no geographic variation when expressed as a ratio of size, could be interpreted as manipulation of evidence. It is possible that I have, in effect, disposed of those characters that showed concordance in variation. However, none of the characters so discarded showed significant geographic variation of any sort, when treated as a function of condylobasal length, which I believe they should have done if they showed more than expression of size. There is also the possibility of correlation between any of the remaining characters. No tests were made for these.

Averaging slopes to get a single picture of variation introduces error. That present in mapping single characters is added to the error arising in fitting isarithms to averaged slopes. Further, the

TABLE I

CONVERSION OF WEIGHTED MEASUREMENTS TO ACTUAL MEASUREMENTS (CHARACTER 1), OR ACTUAL MEASUREMENT/CONDYLOBASAL LENGTH (REMAINING CHARACTERS), AND RANGE OF GEOGRAPHIC VARIATION EXPRESSED AS PERCENTAGE OF THE MEAN. MEASUREMENTS IN CENTIMETERS.

	0–.2	.2–.4	.4–.6	.6–.8	.8–1.0	VARIATION
1	7.580–7.782	7.782–7.984	7.984–8.186	8.186–8.388	8.388–8.590	12.5%
2	.179– .184	.184– .188	.188– .193	.193– .197	.197– .204	12.2%
4	.175– .179	.179– .182	.182– .186	.186– .189	.189– .194	9.7%
5	.202– .206	.206– .209	.209– .213	.213– .216	.216– .220	8.5%
7	.0530– .0544	.0544– .0559	.0599– .0573	.0573– .0588	.0588– .0600	13.2%

final map differs (assuming some discordance in character variation) with every combination of characters, and with the particular weight attributed to each. The averaged isarithm is no more accurate a reflection of reality than are its component parts, and is probably less. It does however provide a means of precise expression of interpretation.

There are then, points of weakness in the approach that can be criticized, and which need refinement. I believe however that it assumes little more than does orthodox procedure, and further, describes precisely what orthodox procedure describes only vaguely and inaccurately.

Discussion of the Results

Variation in condylobasal length (Fig. 3) shows a reasonably close fit to the ranges of subspecies mapped on Figure 1. This is not the case however in variation in the remaining characters, where considerable discordance occurs (Figs. 4, 5, 6 and 7). Of these, characters 2 and 4 (rostral width and canine width at base), since they both measure essentially the same skull dimension, show a fair degree of concordance between themselves. They may be correlated with each other, though no coefficient was calculated to test it. Character 7 (upper molar inner moiety length) provides a good means of separating the *caurina* section from the *americana* section. In the *caurina* section, the measurement is considerably larger than in the *americana* section, and it is not surprising to find largest values of this character in the region of intergradation between the sections (southern British Columbia, Idaho and Montana). However, character 5 (bulla length), which also serves to distinguish the sections, but which is smaller in the *caurina* section than in the *americana* section, also gives largest values in the zone of intergradation. Possibly bulla length displays a form of character displacement (Brown and Wilson, 1956).

The averaged slopes of characters (Fig. 8), when mapped, indicate rate of character change. "Ridge lines in the topography . . . indicate zones of relative disjunction, and so mark natural boundaries for the [delimitation of] geographic varieties. . . . For uniformity, mean values for populations in the lower one half, by area, of each valley or basin . . . are accepted as typifying their groups." (Womble, op. cit.). It will be seen from Figure 8 that geographic populations that could be named by Womble's criteria, fit the current subspecies distributions only approximately. *M. a. actuosa* occurs in an area of change, not of uniformity. *M. a. abietinoides, atrata,* and *americana* are reasonably delimited, but *abieticola, kenaiensis* and *brumalis* only approximately so. The location of type localities fits the distribution of uniform and nameable populations even less adequately.

It appears that there is general discordance of character variation in the characters considered. Condylobasal length gives the best fit to the distribution of subspecies as they are currently accepted. I think that this is to be expected, since examination of the literature shows that most subspecies were described on the basis of size and colour of pelage. I made no quantitative study of fur colour, but it is my impression that size and colour may vary in approximate concordance.

Discussion and Conclusions

It is becoming apparent that the class of entities called subspecies is purely a matter of definition (Gilmour, 1940; Burma, 1954; Clark, 1956; and others), and is as Lotka (1925) has pointed out, defensible as a systematic tool only on grounds of utility. Unfortunately there has been a fashion in the past to treat the definition as accepted fact, and assuming its reality, to look in nature for its evidence. The result has been an inversion of scientific method, in that the describing of subspecies has preceded the study of variation.

Wilson and Brown (1953) and Brown and Wilson (1954) have concluded that where inductive methods replace deductive methods in infraspecific systematics, evidence of discordance of character variation becomes apparent. Examples of discordance have been given by them, and by Petersen (1949), Gillham (1956), and others. Wilson and Brown conclude on these grounds that the subspecies concept, having lost any usefulness that it may have once had, should be abandoned.

In marten, there appears to be a general discordance of variation in some characters at least, and demonstrable perfect concordance is apparently absent. If a single character, size (and possibly a second, colour), is used as a criterion however, a fair fit to variation as described by subspecies results. There is no logical reason why a single character (or pair of them) should not be acceptable in the delimiting of subspecies, so long as the resulting classification is useful in one way or another. I think, however, that there is no biological reason why size and colour should be given precedence over other characters. To do so is to be arbitrary. The results depend on the suppression of other equally relevant evidence, and are therefore useful only to students of size and colour. I am led to conclude that the subspecies concept as currently applied to the *americana* section of marten bears little relation to reality as it concerns the animals, and that it is therefore of little use in studying their variation.

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EDWIN M. HAGMEIER is in the Department of Biology, University of New Brunswick, Fredericton, Canada. Most of the work preliminary to that reported here (including examination of specimens) was done while the author was a student at the University of British Columbia. All subsequent work was done at the University of New Brunswick while he was holding a National Research Council of Canada fellowship.

Geographic Variation and the Subspecies Concept in the Collembolan *Entomobryoides guthriei*

KENNETH CHRISTIANSEN

DETAILED studies of geographic variation in the members of the order Collembola are almost unknown. The closest approach to this type of work has been the study of color forms of *Sira platani* by Handschin (1926), and the various incidental notations in monographic works such as Stach's classic on the Isotomidae (1947), or Delamare's work upon termitophiles and myrmecophiles (1948). It is essential to understand the nature of the geographic variation within the group if any valid judgment is to be made concerning the real value of the various criteria now used to distinguish species in the Collembola. In the work described below an attempt was made to study all visible geographic variation within one species.

One of the major objections which has been raised concerning the subspecies concept is that it is applicable in most cases only if a small part of the total of geographically variable characters is considered. According to this view the net result of the general acceptance of the subspecies idea is to overemphasize the most obvious type of geographic variation while inhibiting the study of the more complicated types. The evidence presented below supports such a thesis, because subspecies of *guthriei* could be created with little difficulty on the basis of any one of several geographically variable characters. However, when an attempt is made to study all characters exhibiting visible geographic variations within the species, any attempt to utilize the subspecies leads only to confusion.

Entomobryoides guthriei is the commonest species of the genus in the western part of North America. It is common over the whole of the western third of the United States from the eastern foothills of the Rockies to the west coast. Scattered populations are found east to the Mississippi. It can readily be distinguished from the generally eastern *E. purpurascens* by differences in the external labial setae, by the male genital plate, and in some cases by the coloration and the structure of the ungues (see Fig. 1). *E. guthriei* is primarily an animal of the middle elevations, although it may occasionally be found as high as 13,000 feet, or more rarely on the slopes of low lying hills. The ecological factors controlling its distribution are still unknown. Throughout its range it displays considerable variation. To obtain some idea of the characters likely to display geographic variation, three populations—one from South Dakota, one from New Mexico, and one from Colorado—were studied exhaustively. This study re-

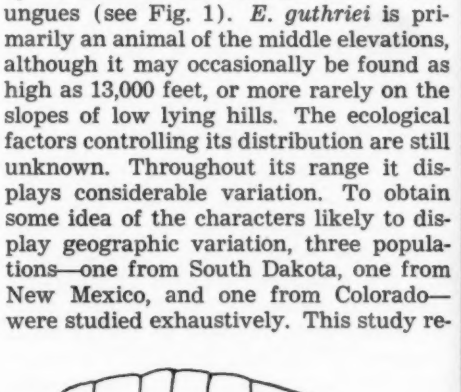

FIG. 1. Outline drawing of *Entomobryoides guthriei* showing location of some of the organs considered. *a*, labial seta; *b*, unguis; *c*, mucro; *d*, genital plate (in male).

vealed fourteen characters which might vary geographically. Further study of these, using all available material, indicated that five characters actually show striking geographic variation. These are: (1) the male genital plate, (2) color and pattern, (3) structure of the mucrones, (4) structure and size of the elements of the ungues, and (5) size of the elements of the labial appendage. The nature of the available samples and of the characters studied resulted in a variety of sample numbers and sizes. The male genital plate is visible only upon a few specimens of any population and is often not visible on any member of a small population. A record for this character may rest upon a single specimen, although the uniformity seen where a large number of males are available makes this a less serious drawback than might be suspected. The color is readily visible upon all specimens in reasonable condition, and the data concerning this character are based on the largest numbers both of populations and of individuals; some samples consist of thousands of specimens. In a few cases where the method of preservation was unknown the populations were eliminated because of the possibility of color change through preservation. The mucronal shape also was readily visible and this was studied in many specimens.

The remaining characters required quantitative treatment. Fourteen populations covering the range of the species as adequately as the available samples would permit were selected for this study. Because of the difficulties involved in measuring, and the small size of most samples, ten specimens were usually measured from each sample. On some occasions where a larger sample was available and a point was in question, ten additional specimens were measured. The specimens were mounted singly in lactic acid so that they could be rotated by gentle pressure upon the coverslip. The measurements were taken with the alignment of the organ being carefully checked to prevent distortion through angle of viewing. All measurements were made with an ocular micrometer. Information concerning the treatment of the data can be seen in the description of the quantitative characteristics.

Geographically Variable Characters

Color and Pattern. The animals vary in color from pale gray (almost white) through pale brown, dark gray, pale and dark blue, to a purplish brown color that is always dark. Although intermediates do occasionally occur it is possible without much difficulty to classify any individual into one of the above classes. If this is done it is found that about two-thirds of the populations consist of one color form alone, or (in very large populations) of one form, with one or two specimens approaching another color. From Figure 2 it can be seen that there are no sharp lines setting off the distribution of various forms. Each of the color forms occupies a lesser range than that of the species, but with one exception no color occupies a very large area to the exclusion of other body colors. The exception is the pale blue form which is the only form found in the northeastern portion of the range. Another point of interest is that the region of greatest color variation (Colorado and northern New Mexico) is also the main area for the most limited of all colors, brown. To a certain extent three overlapping areas can be seen; a southern and western area with purple and dark blue dominant, a northern and eastern region with pale blue dominant, and a central eastern region with brown and gray dominant.

In addition to the colors mentioned above the animals frequently possess more or less striking dark lines upon the posterior borders of the body segments. For purposes of classification the patterns can be broken down into forms having faint lines on the posterior borders of all segments, those having striking lines on the borders, those without darkened lines, and a relatively few populations where

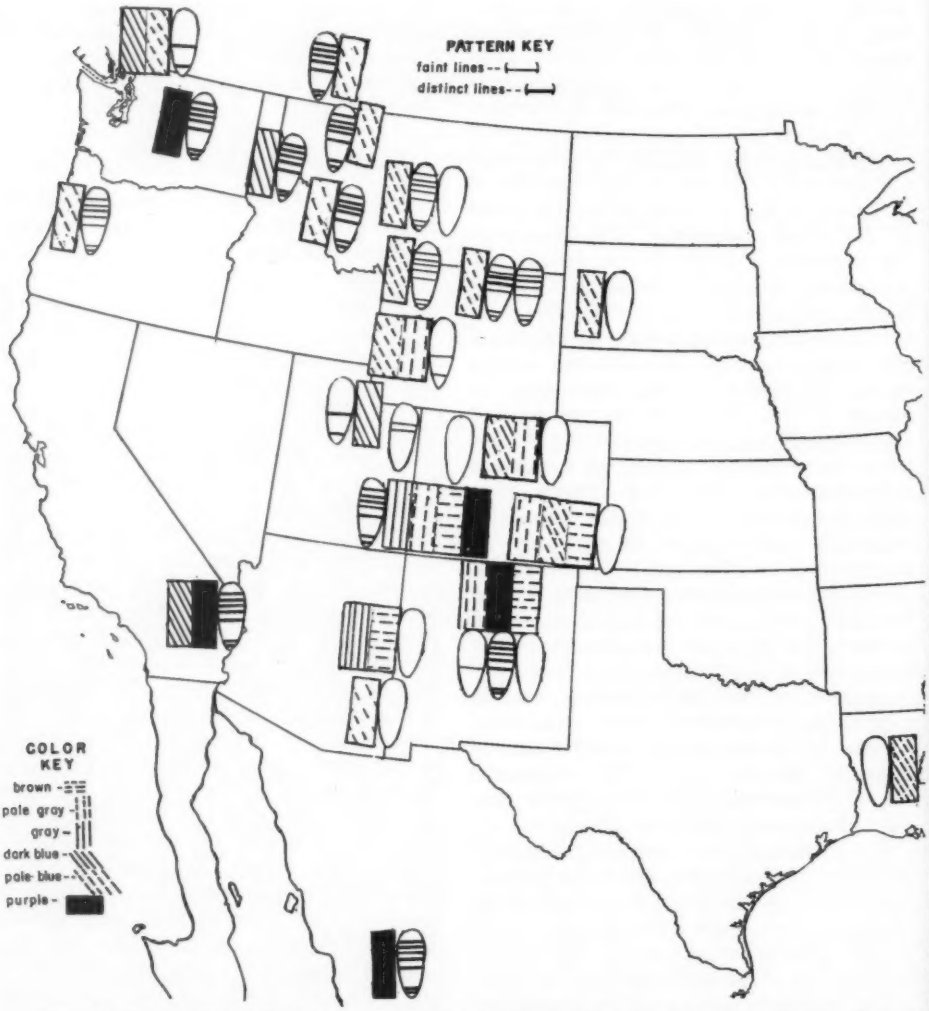

FIG. 2. Geographical distribution of different colors and patterns in *E. guthriei*. The rectangles represent the colors while the ovals represent the striping along the posterior margins of the body segments. Symbols actually touching each other represent members of the same apparent population.

lines occur on only a few of the segments and are absent on the others. The ovals on Figure 2 indicate the distribution of these various patterns. It should be noted that while one pattern prevails in most areas, in a few cases two or more different patterns occur in the same region. As with body color, there is much overlap among abdominal marking patterns. There are a few areas of correlation between color and pattern. For example, all purple forms are heavily striped. How-

ever, there is no general congruence between the distribution of this striping and that of the color of the animals.

Male genital plate. The species is unique among its congeners in the variation in the male genital plate (see Figs. 1 and 3). The three forms commonly occurring in this organ occupy separate ranges and, in one case, show a definite region of intergradation. The three forms appear to occur as a stepped cline from the northwest to the southeast.

Mucronal shape. The shape of the mucro is highly variable in *E. guthriei* (see Figs. 1 and 3). Unlike the male genital plate the different mucronal forms

FIG. 3. Geographical distribution of the various genital plate and mucronal types in *E. guthriei*. Symbols joined with a line represent intermediates between the types illustrated. Symbols encircled represent two types occurring commonly in the same apparent population. Genital plates vary in number of expanded basal setae.

show many intermediates between the various types. It is possible to classify the mucrones into three basic types, although there are some occasions where a specimen can be placed only with difficulty. The types are: first, a form with a large anteapical tooth and the apical tooth projecting forward (type *A*); second, a form with a very small anteapical tooth and the apical tooth projecting upward (type *B*); and last, a type with the two teeth subequal and both projecting upward (type *C*). In most populations all of the adult animals have basically the same mucronal form, or the exceptions make up less than five per cent of the total. In a few populations there are two mucronal types existing in considerable numbers, and these are indicated on the chart (Fig. 3) by two letters encircled. Again the three forms occupy essentially separate ranges, but there is considerable overlap, and a few anomalies (namely, Graham, Arizona). Nevertheless, the three areas blocked out are: (1) a central band running from the northwest to the southeastern portion of the range, (2) a southwestern, and (3) a northeastern sector. These are quite different from the areas mapped out on a basis of genital-plate variation, or those seen in the color patterns.

Labial appendage and ungues. In these characters the differences concern body size and comparative size of different organs, and in some cases the relationships among the various parts vary greatly with age. In view of this it was essential that the data be treated in some fashion which would eliminate or minimize the statistical effect of variations in the size of individuals in the different samples. The various measurements were plotted against total body length (as indicated by the length of an isoauxetic organ) or the different organs and organ parts were considered only in relation to each other. This practice, of constructing ratios, combined with the dependant nature of the various variables made statistical treatment difficult. However, by visual examination alone the data showed such consistency that I felt that the nature of the geographic variation found in this group could be discovered without recourse to sophisticated statistical methods.

The simplest method for comparing the data was to plot size of any organ against the total size of the animal and consider the difference between populations. Varying degrees of intersegmental contraction and varying death poses make total body length an unsatisfactory measurement. Preliminary study indicated that the third tibiotarsus was nearly isoauxetic with body size and this was used instead of body size. In a number of comparisons of two populations the ratios showed no overlap (see Fig. 4). In most comparisons, in spite of overlap between the populations, some differentiation could be observed. However, the differences show no clear geographic patterns, as was true for some of the previous characters.

A second method of treating these data was to compare in the form of ratios the various organ parts of the specimens of different populations. Once again while a clear separation could be observed among many populations, the general picture was one of more or less random differentiation with no clear isolation of separate geographic areas, or the development of clines.

In order to get a broader picture of the nature of this variation, a measure of the average differentiation indicated by the four ratios showing the greatest geographic variation was obtained. The following graphs were made for ten large populations: (1) length of first ungual claw against body length, (2) length of differentiated labial seta against body length, (3) length of differentiated labial seta against attached papilla, and (4) first ungual claw against second ungual claw. In each of the above plots the amount of overlap between each pair of the ten populations was taken up to 70 per cent. An index was prepared indicating the amount of separation between any two populations indicated by these characters. The first use of this index was to dis-

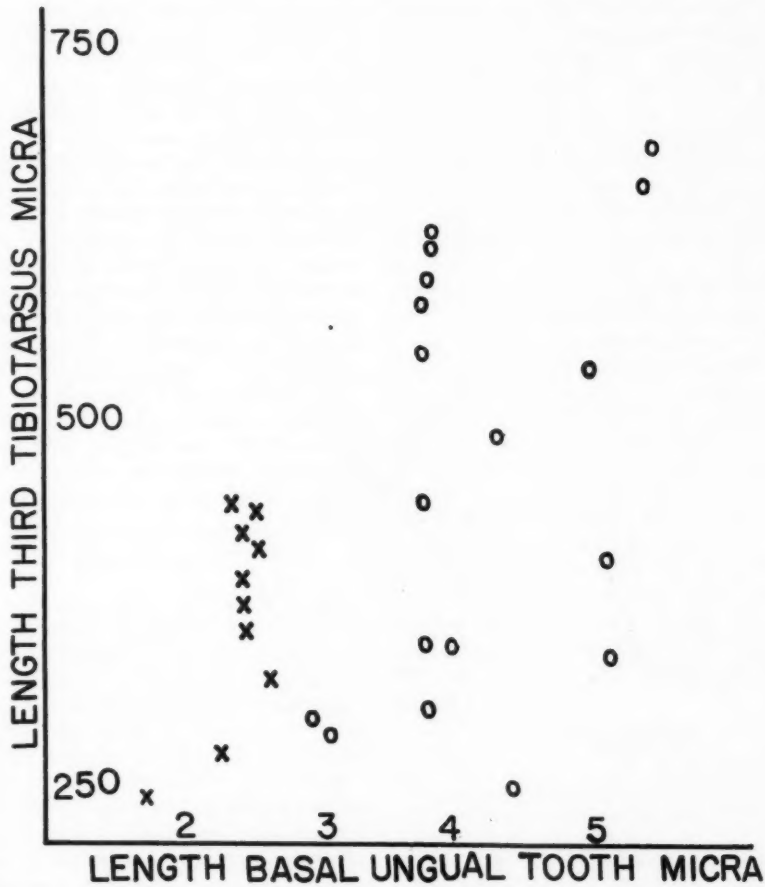

FIG. 4. Total body length as indicated by third tibiotarsus length plotted against length of ungual tooth in two different populations. Each "O" represents a specimen from a locality six miles south of Rapid City, South Dakota. Each "X" represents a population from Manzanilla, Mexico.

cover any correlation existing between the differentiation on this basis and the geographic distance between the populations. The correlation coefficient thus obtained was .36, which is significant at the five per cent level.

A second question concerned any geographic relationships indicated by this data. To indicate these, the ten localities studied were plotted on a map (Fig. 5). In every case where the index was above 240 (average overlap greater than 60 per cent), the localities were joined by a thin line, while a heavier line was used to indicate all those cases where the index was 280 or greater (average overlap greater than 70 per cent). The attributes of the quantitative characters mentioned in the earlier paragraphs can be seen clearly here. A further noteworthy item is the fact that the Utah population (Butterfly Lake) shows a close similarity to several Colorado populations and is clearly differentiated from the South Da-

FIG. 5. Geographical relationships as indicated by four ratios. Solid heavy lines indicate greater than 70 per cent overlap in the ratios between the indicated populations. Solid light line indicates between 60–69 per cent overlap in the ratios between the two populations.

kota population. This is the reverse of the situation found in most of the other characters considered. Generally, the quantitative characters show a picture of striking local differentiation with no larger geographic pattern.

A few minor points of interest were noted in connection with other treatments of data. The ratios of the various organs considered did not for the most part vary strikingly with the size of the animal. The maximum coefficient of correlation found for these was .34, which was below the five per cent level of significance. In two populations, however, this was not the case. In both there was a striking change with growth, producing a correlation coefficient of about .80. These two popula-

tions were the South Dakota and Utah groups, which had, on a basis of the quantitative index, very little similarity.

A last point of interest concerns the only two ratios showing striking differentiation (ratio of first and second ungual teeth, and ratio of external labial seta to attached papilla). In an attempt to understand these the average ratio was compiled for each population. No clear regional unity existed in either series of ratios separately. If, however, the two series of ratio averages were plotted against each other, one as the abscissa and the other as the ordinate on a graph, then it was possible to separate three major geographical areas: a northern, a central, and a southern population. These areas do not correspond to natural ranges of other geographically differentiated characters.

Conclusions

It is apparent that considerable geographic variation of diverse types occurs within the species. This variation may be of a uniform, well-organized type in which one character replaces another, with a narrow zone of intergrade (the male genital plate). It may be of an irregular but basically clear type where each character occupies a large range, with much overlap between ranges, and with many variants occurring in one locality (color). Or it may be of a local, irregular type in which, although many populations can be readily distinguished from each other, there is no clear separation into zones, ranges or clines (the quantitative characters). In this case there is a rough but incomplete correlation between the degree of difference and the distance between the populations concerned.

Any two populations may share a number of characters. Thus the southern California and Mexican populations both have purple striped bodies, mucronal type *A*, and genital plate type 2, while the populations from eastern Colorado and Louisiana are pale, unmarked, and have mucronal type *C*, and genital plate type 3. Any attempt to fit these differences into the classic subspecies pattern produces the dilemma of widely different boundaries for the different characters. If the populations are divided on a basis of the striping you find a southeastern unmarked group and a western striped group, while in coloration the distinction, although vague, is between a southern purple, gray, and brown group and a northern blue group. The picture is further complicated if we add to this the genital plate which divides the species into northwestern, central, and southeastern groups, while the mucronal division into northeastern, central, and southwestern groups adds the final note of confusion. Thus while one character might be a reasonable basis for division into subspecific units this would only be descriptive of one small aspect of the total geographic variation in the species.

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On the Origin of the Eumetazoa

EARL D. HANSON

THE predominant theory for the origin of the Eumetazoa[1] holds that multicellular animals arose from the integration and subsequent individuation of a protistan colony. However, another less widely held and less well documented viewpoint has persisted. This second theory maintains that multicellularity arose through the internal subdivision, by cellularization, of a multinucleate or syncytial protistan individual. Renewed interest in broad phylogenetic problems (Hyman, 1940 and 1942; Hadži 1953; de Beer, 1953; Carter, 1954; Jägersten, 1956) and much new information, especially relating to the Protista,[2] justify a reinvestigation of these ideas. It is the purpose of this paper to determine the adequacy of the theories of eumetazoan origins, to delimit the unresolved problems, and to formulate conclusions which are consistent with our present knowledge.

The paper is divided into three parts. First, the two phylogenetic theories are briefly presented in terms of the biological forms which have been postulated for each, in order to evolve the transition from the Protista to the Eumetazoa. Next, there is a detailed evaluation of the evidence for and against the theories so formulated. The conclusions from this critique (*a*) do not support the theory of colonial integration, and (*b*) point up several difficulties in the proposal arising from the theory of cellularization of the individual. An attempt is made to resolve these latter difficulties. Finally, a phylogeny joining certain protozoans[3] and the Eumetazoa is proposed and the origin of multicellularity in groups other than the Eumetazoa is briefly discussed.

The Two Theories of the Origin of the Eumetazoa

Colonial integration. Haeckel (1874) was the first to work out a detailed application of the principle of colonial integration. On the basis of evidence for recapitulation, and the discovery of germ layers, Haeckel evolved the logically attractive gastraea theory. He postulated that the phylogenetic series

hollow, flagellated, green algal colony → hypothetical blastaea → hypothetical gastraea → diploblastic forms

was recapitulated by the ontogeny

blastula → invaginated gastrula → diploblastic adult.

[1] By *Eumetazoa* we mean the multicellular animals exclusive of the Parazoa (Porifera) and also the Mesozoa if the postulated origin of these latter from the platyhelminths is considered as still unresolved.

[2] Haeckel's term *Protista* is used here as synonymous with *Protoctista*, which has priority (Copeland, 1956). However, in conformity with the recommendations of the International Commission on Zoological Nomenclature (1953) we retain the former word because of its far more common usage.

[3] The term *protozoa*, in this paper, simply refers to animal-like protistans—that is, protistans fundamentally lacking photosynthetic pigments, forming glycogen as their carbohydrate reserve, never organized into hyphal filaments—and designates no taxon. The fact that the organisms grouped under this term are polyphyletic (Grassé, 1952; 1953) is held as justification for abandoning the use of protozoa as a taxonomic category. (Mayr, Linsley and Usinger, 1953).

His contemporaries pointed out that the hollow gastrula is not found in the ontogeny of the primitive Metazoa, i.e., the coelenterates. Rather, a solid stereogastrula is the primitive gastrula and entoderm is formed, not by invagination, but by inwandering of surface cells in most cases (Metschnikoff, 1887) and occasionally by delamination (Lankester, 1877). Hence, the hypothetical gastraea was thought to be improbable on ontogenetic grounds. Utilizing these criticisms Hyman (1940) has formulated the following modification of the Haeckelian viewpoint.

> We may regard it as plausible to suppose that the Metazoa arose from an axiate hollow spherical flagellated colony in which there occurred first a differentiation into somatic and reproductive cells and then a differentiation into locomotor-perceptive and nutritive types, through the wandering of the latter into the interior. In this parenchymula or stereogastrula, food was caught by the surface cells in protozoan fashion and passed into the interior amoeboid cells for digestion. The sex cells were also relegated to the interior and received food supplies from the amoeboid cells, as still happens in many lower metazoans. The anterior pole probably bore special sensory cells or a tuft of sensory cilia. Such an organism may be considered the common ancestor of the coelenterates and flatworms. (By permission from *The Invertebrates,* vol. I, by L. H. Hyman. Copyright, 1940. McGraw-Hill Book Company, Inc. p. 252)

Hyman proposes that the coelenterate planula is the present-day form most closely resembling this hypothetical common ancestor.

Naef (1921) and Jägersten (1956) do not accept the stereogastrula modification of Haeckel's gastraea. Naef proposes this evolution:

blastaea ⟶ progastraea ⟶ gastraea ⟶ metagastraea ⟶ primitive metazoan.

The gastraeal forms are bottom crawling. In the progastraea feeding-cells accumulate at the organism-substrate interface. In the gastraea there is invagination of the feeding surface and in the metagastraea the invagination achieves a permanent, primitive archenteron. Jägersten adds the qualification that the gastraea is bilaterally symmetrical, instead of being radial.

In spite of these different opinions regarding the hypothetical primitive metazoan, there is, however, agreement that the protistan ancestor closely resembled a colonial volvocine member of the Chlorophyceae. In support of this flagellate ancestry Hyman (1940) presents the following arguments, which may well represent the views of all the Haeckelian evolutionists. (1) The flagellates represent a highly plastic group from which in all probability the entire plant kingdom and the Protozoa have originated. (2) The flagellates show a great tendency toward the formation of compact colonies, and, of these, the spherical ones resemble stages in the embryonic development of the Metazoa. (3) Moreover, in some of the Volvocales such colonies show a high degree of individuation, including both anatomical and physiological differentiation among members of the colony. (4) The almost universal occurrence of flagellated sperm in the Metazoa is cited as evidence for the descent of the Metazoa from the flagellates.

Other professedly non-Haeckelian phylogenies embodying the principle of colonial integration have been put forward. Lameere (1901, 1908) has suggested that colonies of collared flagellates gave rise to the sponges which in turn gave rise to the eumetazoans. Such a view does not accord with the present-day consensus that the sponges are clearly a side branch of invertebrate evolution. This suggestion will not be considered further here. Hardy (1953) proposes that the Metazoa originated "from relatively simple metaphytes." But in so far as these metaphytes are described as "spherical volvox-like," we fail to see that this differs in any essential aspect from the Haeckelian view.

Cellularization of an individual protis-

tan. All proponents of this view (von Jhering, 1877; Kent, 1880–1882; Sedgwick, 1894; Hadži, 1953; de Beer, 1954; Pax, 1954) agree that a multinucleate ciliated protozoan is the most probable hypothetical ancestor of the Eumetazoa. Metschnikoff (1886) criticized the proposals of von Jhering and Kent by pointing out the following. The fertilized insect egg develops into a multinucleate syncytium. Application of the biogenetic law would mean the insects are the most primitive Metazoa. Metschnikoff concludes that since this phylogeny is untenable, the original proposal is also untenable. This argument is still considered to be of major importance by present-day opponents of the cellularization hypothesis (Jägersten, 1956; Sachwatkin, 1956). Except for a general critique of the biogenetic law (esp. de Beer, 1940), Metschnikoff's criticism has never been explicitly answered, neither by those who more recently have supported von Jhering and Kent, i.e. Hadži, de Beer, and Pax, nor by those (Dobell, 1911; Dogiel, 1927) who, on broad theoretical grounds, have favored the principle of cellularization as opposed to integration. We shall return to Metschnikoff's point later.

Hadži's proposals, albeit brief, are the most detailed formulation of the cellularization hypothesis.[4] He proposes: The Coelenterates are derived from rhabdocoeloid forms. These, in turn, are evolved from acoeloid flatworms. The Acoela are, therefore, the most primitive Eumetazoa, and they evolved from "Infusoria [=Ciliophora] -like ancestors."[5] These hypothetical Infusoria-like forms are similar to present-day ciliates except that they lack the macronucleus.

The evidence Hadži gives in support of his views is the following. (1) The correct interpretation of the biogenetic law would necessitate postulating the origin of the Eumetazoa from an individual and not from the integration of many individuals. (2) ". . . the respective structural parts of both animal types [the ciliates, exclusive of their nuclei, and the acoels] are not only outwardly similar but in reality homologous. This applies especially to the three somatic layers . . . ," to the ciliate contractile vacuoles and turbellarian protonephridia, and to the ciliate trichocysts and the turbellarian sagittocysts. (3) Furthermore, cellularization is at a minimum in the Acoela. The germ cells are cellularized, but the rest of the body is a multinucleate, highly organized mass of protoplasm without cell membranes. (4) There is a size-range overlap in the two groups. (5) A striking similarity exists between the ciliates and acoels in the fact that both groups are hermaphroditic and evince crossfertilization. That is, each member of a conjugating or copulating pair, as the case may be, produces male and female gametic material and the male gametic material of one partner fuses with the female gametic material of the other partner. In sum: the protistan ancestor is a hypothetical,

[4] The major source of Hadži's views has been his 1953 paper. This is not, however, the only place where Professor Hadži has dealt with the origin of the Eumetazoa. Unfortunately for this author the other papers are published in Serbian with English summaries. The point to be made here is that certain topics touched on in the present paper, e.g. the importance of *Calonympha* and *Opalina*, have been treated independently by Professor Hadži. The author is indebted to Dr. Marko Zalokar for the opportunity of examining certain of Professor Hadži's reprints.

[5] Kent, Vol. II p. 475, in a footnote to his discussion of this topic has the following interesting statement. "The near approach of the Ciliate Infusoria to the structural plan of the lower Turbellaria is briefly indicated by Professor [T. H.] Huxley at p. 678 of his 'Anatomy of Invertebrated Animals,' ed. 1887; while, in a communication addressed to the author since the publication of the first two parts of this Manual, this authority has more distinctly referred to the Holotricha as probably constituting, in a connection with the Turbellaria, the proper line of evolution from the Protozoic to the Metazoic series." Furthermore, certain investigators, e.g., Schmidt (1849) and Diesing (1865–1866), in recognition of morphological similarities, proposed that the ciliated Infusoria be classified with the lower worms or as "Prothelmintha!"

ciliate-like, multinucleated protozoan; the primitive metazoan is a partially cellularized acoel. Quite general developmental and some specific morphological and sexual phenomena constitute the argument for this phylogeny.

Critique of the Theories

This section evaluates the theories summarized in the previous section. General comments on methodology are followed by the critique itself.

Methodology. In dealing with phylogenetic problems at the level of the highest taxa, it must be emphasized, as Carter (1954) has stated, that the evolutionary separation of these large groups antedates the fossil-bearing rocks of today, and much change has occurred since that time. The use, therefore, of present-day forms to reconstruct pre-fossil animal history of hundreds of millions of years ago must be submitted to the most searching scrutiny. Nevertheless, the transition from the acellular or unicellular to the cellular or multicellular is so important a step in the evolution of higher forms, that it is imperative attempts be made to comprehend it, however inadequate our information may be. For only in attempting can deficiencies be crystallized and directions for new work clarified.

The analysis of phylogenetic relations is a comparative process. All the relevant aspects of the *biology* of the pertinent forms must be compared, not just their developmental biology or morphology, for it is a truism that an organism is not solely ontogeny or shape. The whole organism is involved in evolution and not simply this or that aspect of it. Where there are no fossils, living forms are the only concrete forms we have from which to infer the biology of our hypothetical forms. Hence, in the present case, we will be comparing the biological characters of the forms most closely resembling the postulated primitive eumetazoan with the biology of the organisms most similar to the postulated ancestral protistan. That is, concerning Hyman's proposal, we compare the coelenterate planula and the volvocine colonies. For Jägersten's views no extensive biological comparisons are possible since the bilaterogastraea of that author is described in idealized morphological terms and no present-day form is suggested to typify its total biology. Nor, indeed, could such be suggested, since Jägersten utilizes eleven hypothetical modifications of this bilateral, invaginated gastrula to give rise variously to the sponges, Mesozoa, Cnidaria, Ctenophora, Deutero- and Proterostomia. For Hadži's views, we compare the Acoela and the Ciliophora, excluding, in the latter, the macronucleus for reasons given on page 18.

In such comparisons we are interested only in the fundamental characters of the derived form. It must be determined how many of the fundamental characters of the eumetazoan are found also in the protistan; [6] and, how many of these characters, if not held in common, are readily derivable from the protistan form. What is a *fundamental character?* What is meant by *readily derivable?* By *fundamental character* is meant a character of widespread occurrence in the group under examination. The term *readily derivable* implies that few genetic mutational changes are necessary to pass from one character to another.

The biological comparisons are divided into the following categories: external morphology, internal morphology and histology, cytology, developmental biology, comparative biochemistry and physiology, ethology, and ecology. This listing essentially follows the categories of taxonomic characters suggested by Mayr, Linsley and Usinger (1953), except

[6] It would be preferable of course to ask: How many of the fundamental characters of the eumetazoan can be homologized with characters found in the protistan? However, as will be seen in the following pages, the present state of developmental and morphological knowledge in this area is not sufficiently refined to be useful for rigorous deduction of homologies.

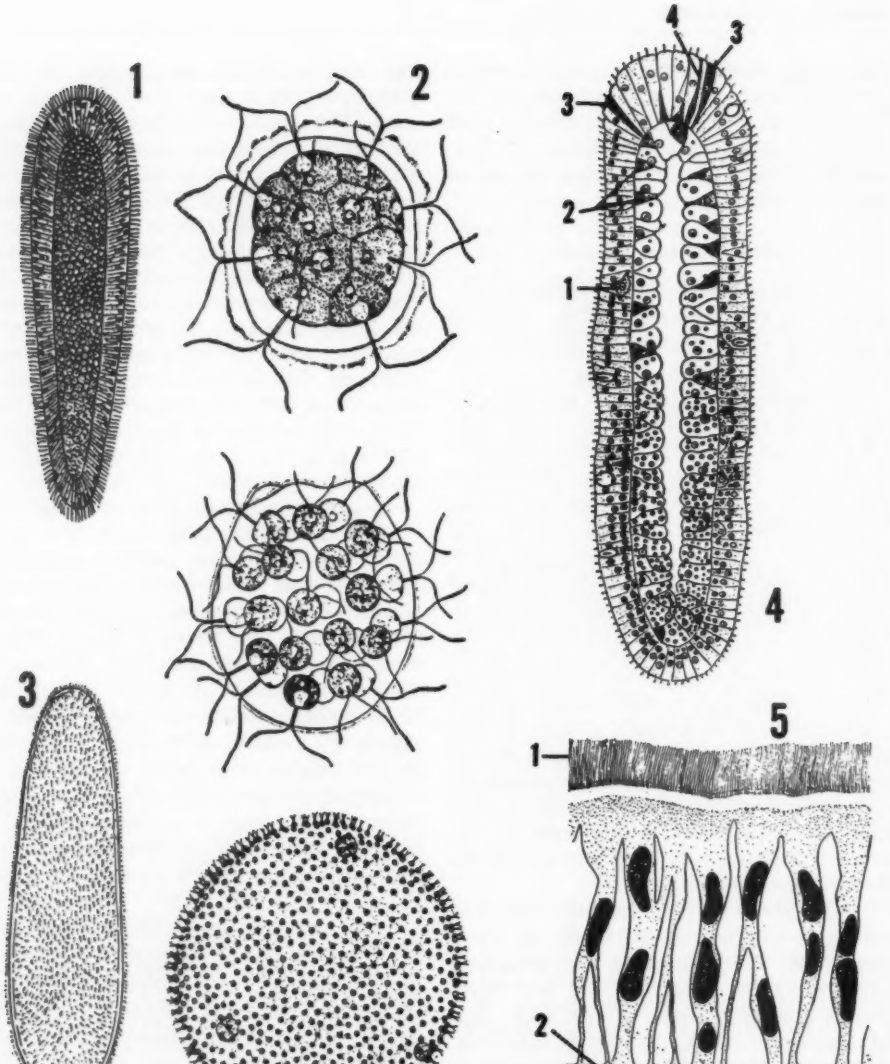


FIG. 1. Planula larva, from life. (By permission from *The Invertebrates, Vol. I,* by L. H. Hyman. Copyright 1940. McGraw-Hill Book Company, Inc.)

FIG. 2. Representative volvocine coenobia. *Upper, Pandorina morum* Bory (approx. 650 ×). *Middle, Endorina unicocca* G. M. Smith (approx. 435 ×). *Lower, Volvox aureus* Ehrenberg (approx. 200 ×). (From G. M. Smith, 1938).

FIG. 3. The acoel *Afronta aurantiaca,* from life. (By permission from *The Invertebrates, Vol. II,* by L. H. Hyman. Copyright 1951. McGraw-Hill Book Company, Inc.)

for the omission of genetic characters. The impossibility of obtaining breeding data here is obvious. Before proceeding it should be noted that the planula is a larval form. Its morphology and embryology are sufficiently well known to permit careful comparisons between it and the flagellate colony in these respects. But for certain other of its biological characters, e.g., nutrition and ecology, inferences from adult forms must be used.

Except when otherwise noted the information in the following pages is taken from Kahl (1931), Bresslau (1935), Smith (1938), Hyman (1940, 1951), Grassé (1952), and Kudo (1954).

External morphology. Planulae are morphologically radially symmetrical[7] and ovoid. The size varies from roughly one half to two millimeters. These larvae are completely and uniformly covered with cilia. No other external structures are present (Fig. 1).

The colonies of the Volvocaceae are radially symmetrical, more spherical than ovoid, and of a size range similar to that of the planulae. All colonies possess a definite number of cells arranged in a specific manner. That is, they are coenobia. There is a gelatinous sheath around all cells. Each member of the flagellate colony possesses two flagella as long as or longer than the length of the cell bearing them (Fig. 2).

The similarities between the external morphology of the planula and the volvocine coenobium are thus seen to be of a very general nature: both possess radial symmetry and are of roughly the same size and shape. The differences relate to locomotory structures and arrangement of the cells. Fine structures of flagella and cilia are identical. In cross-section both have an outer membrane, then a ring of nine fibrils, and centrally two more fibrils (Bradfield, 1955). Even though the flagellum is usually longer and more flexible than the cilium, there is probably no unbridgeable difference between a flagellum and cilium. What may be important, however, is the number of the locomotory structures found in each cell, since flagellar number is an important aspect of protistan organization. Hence the question: Is each cell of the planula bi-ciliated? There appears to be no work which supplies the answer except for the early work of Metschnikoff (1886) in which there is depicted a single cilium for each cell. The planula is not organized with a definite number of cells as are the volvocine colonies. That is, it is not coenobial. Neither is there a gelatinous sheath around each planula cell. These aspects of volvocine organization have no counterpart in the planula.

The Acoela are bilaterally symmetrical, of somewhat varying body shape, but typically ovoid with a dorso-ventral flattening. The body varies in length from commonly somewhat less than one millimeter to several millimeters. It is completely and uniformly ciliated. There is a ventral median mouth which may or may not lead into a simple, ciliated pharynx. A male gonopore is situated in a median, posterior, either ventral or terminal position. When a female gonopore is present it is anterior to the male opening (Fig. 3).

Ciliates are both radially and bilaterally symmetrical. They also show various forms of assymmetry. However, bilateral symmetry is most common. Their size ranges from about one hundredth of a

[7] The swimming behavior of planulae, however, suggests a bilateral symmetry.

FIG. 4. Mature planula of *Gonothyraea. 1,* Ganglion cell; *2,* entodermal gland cell; *3,* ectodermal gland cell; *4,* sensory cell. (After original by Werlfert, by permission from *The Invertebrates, Vol. I,* by L. H. Hyman. Copyright 1940. McGraw-Hill Book Company, Inc.)

FIG. 5. Longitudinal section through the "Deckschichte" or epidermis of the acoel *Polysolenoposthia porsildi. 1,* cilium; *2,* contractile fibril of circular layer; *3,* contractile fibril of longitudinal layer; *4,* fixed nuclei of outer mesenchyme; *5,* dorso-ventral contractile fibril. (Original by An der Lan 1935–1936, by permission from *The Invertebrates, Vol. II,* by L. H. Hyman. Copyright 1951. McGraw-Hill Book Company, Inc.)

millimeter to two or three millimeters in length. The cilia very often cover the entire body but in some the cilia are only in special tufts and membranelles. With the exception of the Astomatida and Suctorida, all possess oral structures which are anteriorly located in forms with radial symmetry, on the plane which defines bilaterality in the bilateral forms, and variously placed in the assymmetrical ciliates. There are many other more specialized structures also present, e.g., egestatory pores, platelets, stalks, tentacles, etc.

General acoel size, shape and symmetry have their counterparts in the ciliates. The presence and placement of the mouth is similar in the Acoela and the bilateral Ciliophora. Ciliation is similar in both. There is no ciliate structure equivalent to the permanent gonopore(s) of the Acoela.

Internal morphology. The planula is typically cellularized and possesses an outer, columnar ectoderm and an inner, solid endoderm. The latter is syncytial only in those few forms carrying large amounts of yolk. Considerable cell differentiation can be present throughout (Fig. 4). Nerve cells, sensory cells, gland cells, muscle fibrils and nematocysts are found. However, it can be argued that the volvocine colony is more comparable to the coeloblastula which ontogenetically precedes the planula. This blastula does not show a high degree of differentiation. The blastomeres of this early stage are loosely associated and seemingly without definite arrangement.

The volvocine coenobium always shows a definite arrangement of the constituent cells (see Fig. 2). The morphological differentiation among these cells pertains to size. The cells appear as larger or smaller editions of the same basic plan. There is also the gelatinous matrix mentioned earlier and almost without exception there is a cellulose cell wall around each cell.

The major contrasting feature of the coenobium and the coelenterate blastula or stereogastrula is that the former possesses a definite cellular arrangement with very little morphological cellular differentiation, whereas the latter is indefinitely organized, except for the body layers, and in later stages exhibits a high degree of cellular differentiation. There is nothing in the coenobium that even vaguely suggests an evolutionary starting point for nerve cells, sensory cells, gland cells, muscle fibrils and nematocysts.

The acoels possess a syncytial epidermoid layer without a basement membrane in many, or perhaps all, forms (Fig. 5). In this connection An der Lan (1935–36) states the following: "Wir sehen also, dass fast alle Autoren die Verhältnisse richtig erkennen und beschreiben, aber immer wieder von 'Zellen' sprechen. Es ist eben so, wie Luther sagt, dass das Plasma der Deckschichte ohne Grenze in dasjenige des Parenchyms übergeht." The endodermal mesenchyme is also syncytial. Moreover, this mesenchyme is differentiated into an outer relatively fixed mesenchyme (Randparenchym of Bresslau) and an inner loosely organized mass (Verdauungs- or Zentralparenchym) in which food vacuoles occur. Nuclei are found in all parts of the body (Fig. 6). Fluid-filled spaces are reported in the epidermoid layer and mesenchyme. The cilia are provided with basal granules, roots extend internally and there appears to be an interconnecting "fibrillar system similar to that of ciliate Protozoa" (Hyman, 1951). Other specialized structures, rhabdoids and sagittocysts, are also found in the epidermoid layer of certain acoels. Sagittocysts are pointed vesicles containing a central, explosively protrusible rod or needle. Rhabdoids are rod-like secretion products of sub-epidermal glands. Glandular structures are common in the Acoela. The eosinophilous glands, which produce the rhabdoids, are scattered sparingly throughout the acoel body. A special group of these makes up the caudal glands which are common among the acoels and perhaps have an adhesive function. The cyanophilous glands are typically found as a cluster of secretory structures in the anterior end of the animal. These frontal

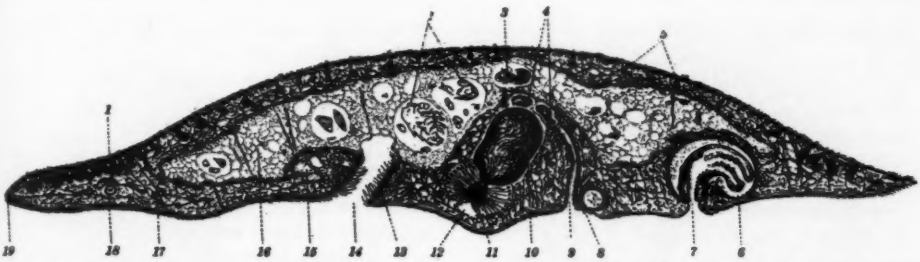


FIG. 6. Dorso-ventral longitudinal section of *Convoluta convoluta*. *1*, brain; *2*, food particle (copepod) in inner mesenchyme; *3*, sperm-packet in inner mesenchyme; *4*, sperm-packet at the end of the vagina; *5*, cyanophilous epidermal gland; *6*, copulatory apparatus; *7*, male gonopore; *8*, muscular paragenital secretory organelle; *9*, female gonopore; *10*, seminal bursa; *11*, sperm duct; *12*, mesenchymal vacuole leading to eggs; *13*, pharynx; *14*, mouth; *15*, outer mesenchyme; *16*, inner mesenchyme; *17*, dorso-ventral contractile fibril; *18*, statocyst; *19*, opening of frontal gland. (From Bresslau, 1933)

glands are thought to be important in food capture. So called multicellular glands, of somewhat dubious status, have been reported only for the acoel genus *Convoluta*. Contractile fibrils (they are not muscle fibers in the sense of groups of specialized cells) are universal in the Acoela (see Fig. 5). In some forms they are in the epidermoid layer, this being considered the primitive condition, but in all others they are just below this layer. The musculature typically consists of the outer circularly arranged fibrils and inner longitudinal fibrils. The sexual organs of the primitive Acoela are very simple. The primitive male system consists of spermatogonia, usually differentiating from a median lateral area of the inner mesenchyme, paired seminal vesicles, and the male canal with or without a thickened muscular wall. The terminal part of this canal is the penis. The female system consists simply of ovogonia. These arise from the inner mesenchyme median to the spermatogonia (Fig. 7). More complicated systems involve seminal bursae, permanent female gonopores and attached vaginae, and complex penes (see Fig. 6). There is a primitive nervous system in the Acoela. In the simplest forms, e.g., *Nemertoderma*, it is located in the epidermoid layer. In the others it is just beneath this layer. The system consists of a net of fibrils, best developed anteriorly, with usually three pairs of longitudinal strands (Fig. 8). There is often a concentration of nerve matter in the region of the statocyst. The statocyst is common to nearly all Acoela as a single or, less commonly, a double, structure located in the anterior mid-portion of the animal (see Figs. 6 and 8). A few forms possess eye-spots. There is no digestive system; food vacuoles are formed within the inner mesenchymal syncytium. There is no excretory system; the protonephridia of the higher Turbellaria are absent.

In comparison the ciliate body is organized into ecto- and endoplasm (Fig. 9). The ectoplasm, bounded externally by a thin membrane, the pellicle, is typically transparent except for the presence of certain organelles to be mentioned shortly. The endoplasm is visibly very complex. Cyclosis is conspicuous in the inner endoplasm due to the motion of food vacuoles, crystals, and granules. The nuclei lie in the inner endoplasm. The position of the nuclei is essentially fixed, especially in those forms containing few nuclei, despite the streaming of the cytoplasm. The number of nuclei in the ciliates is variable. Usually there is one large macronucleus and one to a few micronuclei. Certain forms, however, are multinucleate with regard to both macro- and micronuclei. Contractile vacuoles are usually fixed in position in the outermost part of the endo-

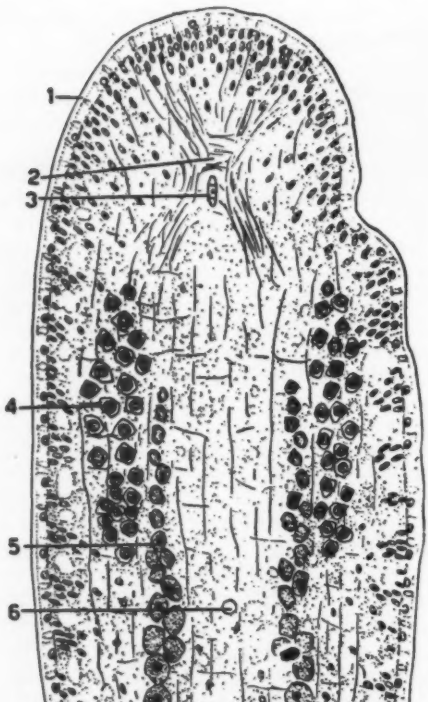


FIG. 7. Lateral longitudinal section of *Afronta* showing simplest reproductive system found in Turbellaria. *1*, epidermis; *2*, brain; *3*, statocyst; *4*, spermatogonia; *5*, ovogonia; *6*, mouth; *7*, seminal vesicle; *8*, penis; *9*, male gonopore. (By permission from *The Invertebrates, Vol. II*, by L. H. Hyman. Copyright, 1951. McGraw Hill Book Company, Inc.)

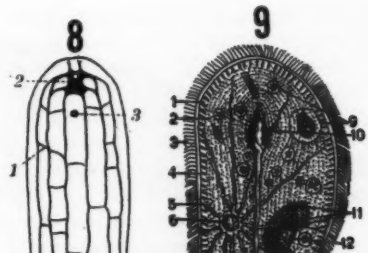


FIG. 8. Nerve net of *Convoluta roscoffensis*. *1*, nerve fibril; *2*, brain; *3*, mouth; *4*, female gonopore; *5*, male gonopore. (Original by Delage, from Bresslau, 1933.)

FIG. 9. The holotrichous hymenostome *Frontonia* as seen from the ventral side. *1*, ectoplasm; *2*, endoplasm; *3*, cilium; *4*, pellicle; *5*, trichocyst; *6*, contractile vacuole; *7*, radial canals; *8*, ciliary row; *9*, food vacuoles; *10*, mouth; *11*, micronuclei; *12*, macronucleus. (After original by Tönniges, by permission from *The Invertebrates, Vol. I.*, by L. H. Hyman. Copyright, 1940. McGraw-Hill Book Company, Inc.)

plasm and have outlets through the ectoplasm to the surface of the organism. Explosive elements, such as trichocysts, are ectoplasmic, as are the ciliary basal granules or kinetosomes and their attached fibrils or kinetodesmas. The same is true for the trichites and poison secreting toxicysts of *Dileptus*, and may be true of the source of the mucous secretions of *Spirostomum* and *Urocentrum* which function adhesively (Jennings, 1923). Contractile elements, other than those in stalks, are quite common in the larger ciliates and lie near the body surface. There are no permanent internal sexual organelles in the ciliates except for the micronucleus which serves as the source of both male and female gamete nuclei. In *Paramecium* at the time of sexual pairing a paroral cone—a cytoplasmic projection—is seen, which marks the point of transfer of the male gamete nucleus from one pair member to the other. Many fibrillar systems have been described in the ectoplasm of

ciliates. Some have been demonstrated by techniques involving the reduction of silver salts. Others are seen by use of basic dyes such as hematoxylin. Müller's vesicles are found in a few gymnostomous ciliates, and are claimed by Penard to be statocysts. Food vacuoles are formed at the inner end of the gullet and circulate, as stated above, in the inner endoplasm. No specific excretory organelles are present, though such a function along with the demonstrated osmoregulatory function has been proposed for the contractile vacuoles which are present in both fresh water and marine ciliates.

On a comparative basis there is much of the internal morphology of the Acoela that has its counterpart in the ciliates. The general lack of internal cell boundaries plus the plurality of nuclei are striking instances. Another is the general body organization into inner central mass with digestive and reproductive structures, and outer portion with locomotor, protective and food capturing structures. Detailed comparisons show, in the case of the reproductive system, that the acoel reproductive structures are far more elaborate than those of ciliates. The paroral cone of *Paramecium* shows certain similarities to the male gonopore of the acoels, in that it is the site of release of male gametic material. It is not known whether paroral cones are widespread in the Ciliophora. There are insufficient data on the fine structures of the acoel sagittocysts, rhabdoids, frontal and caudal glands and their products, to permit a satisfactory comparison with the much more thoroughly known trichocysts, trichites, toxicysts, and mucous secreting organelles of the ciliates. Similarly, no critical comparison is possible yet between the acoel statocyst and Müller's vesicles in the gymnostomous ciliates. The similarities between these structures are as yet only highly suggestive. The acoel nervous system appears to be a system without a counterpart in the ciliates. A crude approximation of a nervous system in the ciliates might be the so-called neuromotor system: in some forms a coordination of ciliary beat is attributed to it, and it is cytologically demonstrable by silver impregnation techniques. In certain holotrichous ciliates the electron microscope has shown it to be formed of discontinuous elements, the kinetodesmas (Metz, Pitelka, and Westfall, 1953; Metz and Westfall, 1954). In the complex spirotrich *Diplodinium ecaudatum*, Sharp (1914) reports a very complicated system of fibers which is clearly not made up exclusively of kinetodesmal sub-units. Fernandez (1949), studying *Ophryoscolex purkinjei* with silver impregnation techniques, also reports complicated structures apparently not associated with kinetodesmas. It is of the greatest interest to know that the neuro-motor system in these forms appears to be phylogenetically homologous to that of the lower ciliates (Kantor, 1956). The acoel nerve net is formed of continuous fibrils which, though peripherally located, are below the level of the ciliary basal granules and their roots. Hyman suggests that the nerve elements of the Acoela have their phylogenetic origin as a nervous accumulation about the statocyst.

In summarizing the preceding paragraphs it is obvious that more information is available on the ciliates and acoels than on the volvocine coenobium and coelenterate larval forms. This is not due so much to a disparity of information as to the fact that the adult forms of the acoels and ciliates show more complexity of internal structure than do the coelenterate blastula and planula and the phytomonad colony. There is little similarity in the internal organization of the latter forms. Nonetheless, it is perhaps not inconceivable that loss of gelatin capsule and of cellulose walls might lead to the indefinitely organized coelenterate blastula. However, it seems unlikely that the limited cellular differentiation of the coenobium is the forerunner of the complex, widespread differentiation of the planula.

Cytology. There is very little informa-

tion regarding the chromosomal morphology, achromatic figure, and cytoplasmic inclusions in the coelenterates. What does exist is useless for critical comparative purposes since neither the text nor the accompanying figures were prepared for cytological purposes. Therefore, instead of trying to interpret, for example, sketchy chromosome and spindle configurations used in a diagram to illustrate cleavage planes, this aspect of the present problem will be omitted except for general considerations. The same will hold for the Acoela.

The coelenterate life cycles have been extensively studied. This work reveals that there is pre-zygotic reduction of the chromosome number. Hence the planula is a diploid form; haploidy is restricted to the gametes. The achromatic figure of the coelenterates is amphiastral (Wilson, 1928). Mitochondria are undoubtedly present (Sharp, 1934). Various specialized cell inclusions are found, e.g., secretory granules in secretory cells, nematocysts in cnidoblasts, etc.

The volvocine phytomonads show post-zygotic reduction divisions. The zygote is therefore the only diploid stage of the life cycle, the coenobium being haploid. The mitotic figure is anastral but a centriole is present. Eye-spots, plastids (usually cup-shaped), pyrenoids, and contractile vacuoles are always present. Food reserves in the form of starch are present.

In comparing these forms cytologically, it is very hard to evaluate differences such as the anastral achromatic figure as opposed to the amphiastral. Not enough is known of the biology of the aster to use it meaningfully in phylogenetic considerations. In terms of cytoplasmic inclusions it is clear that volvocine cells are plant-like. And even the viable colorless forms, which are known *only* among the *uni*cellular forms, retain the ability to store starch. There is a clear-cut difference between the volvocine coenobium and the planula in terms of chromosome cycle. In the former the vegetative form is haploid and in the latter diploid. To account for this difference, there must have occurred a postponement of reduction divisions to allow the development of a diplontic life cycle from the haplontic, a situation that is not unknown in the green algae.

Acoela are diploid forms with haploid gametes. Little is known of the cytoplasmic inclusions. Concrements (pigmented crystalline substances) are found in some forms, located either below the epidermoid layer, or more internally.

Ciliates are diploid, possessing haploid gametic structures. The mitotic apparatus is anastral, acentric (no centrosome or centriole), and the nuclear membrane remains intact during meiosis and mitosis. Inclusions in the cytoplasm include mitochondria, Golgi bodies at least in certain forms (the osmiophilic properties of the contractile vacuoles are not here considered as equivalent to a Golgi apparatus, see Grassé 1952), and crystalline bodies, perhaps of an amino acid nature.

It is a valid generalization that the metazoan achromatic figure is amphiastral and centric. The fact that the ciliate achromatic figure shows neither of these characters is considered by Grassé as mitigating heavily against the Ciliophora as progenitors of the Metazoa. If we assume that the Acoela are typical metazoans as regards their mitotic apparatus, then, indeed there is a sharp difference between them and the ciliates. But two lines of experimental work indicate that this difference is not unbridgeable. Pollister and Pollister (1943) report the origin of centrioles from centromeres in certain gastropods. Wilson (1928) has concluded that cytasters, structurally and functionally indistinguishable from cleavage asters, are formable *de novo*. Harvey (1936, 1940) also reports this. Hence the absence of asters and centrioles in the ciliates does not mean we must expect the same condition in forms thought to be descended from ciliate-like ancestors. The problem of the nuclear membrane—always present in the ciliates, absent in meiosis and mitosis in the Eumetazoa—has a different possible solution. Bresslau (p. 60) states that

in the primitive rhabdocoels, the Catenulidae, nuclear division is conspicuously suggestive of mitosis in the protistan nucleus. A figure is presented in support of this. The most obvious protistan-like feature is the constancy of the nuclear membrane. It might therefore be inferred that since a permanent nuclear membrane is present in the lower rhabdocoels, it is also present in the acoels. Concerning chromosome cycles it is clear that no difference exists between the ciliates and the Acoela. Neither does the meager information on cytoplasmic inclusions show important differences.

Developmental biology. The coelenterates are both monoecius and dioecius. The typically metazoan, flagellated, male gametes and the egg are formed in definite gonadal structures whose location is different in different species. The origin of the sex cells is usually from interstitial cells of either the epidermis or gastrodermis. Fertilization is either internal or external. Cleavage in the Hydrozoa is indeterminate, holoblastic, adequal, and radial. There is apparently little information for the Scyphozoa, while in the Anthozoa the cleavage is holoblastic and commonly equal. A ciliated coeloblastula is typical. The transformation of this hollow blastula into the solid stereogastrula of the planula is achieved by ingression, both uni- and multipolar, also by delamination, and rarely, as in the Corontae, by invagination. Later, when the planula settles down, the solid endodermal mass develops a gastrovascular cavity and other features characteristic of the polyp (see Fig. 4).

The volvocine coenobia show both monoecius and dioecius forms. Sexual reproduction can be isogamous or anisogamous, the latter reaching a clear-cut oögamy in forms such as *Volvox*. The anteriorly flagellated gametes are usually biflagellate. In certain forms all cells of the coenobium become gametes, in others only the cells at one end of the colony are gametogenic. Fertilization is usually external to the colony in all except the oögamous forms. The zygote secretes around itself a wall which is often orangered due to hematochrome. Germination is preceded by reduction division of the zygote nucleus. Biflagellate haploid zoospores are released and colony formation then ensues, exhibiting, in the more complex forms such as *Eudorina, Pleodorina,* and *Volvox,* a precise and unique pattern of cleavage planes (Fig. 10). A hollow ball of cells is produced which has its flagella lining the *inner* surface of this sphere. By inversion—turning inside out through the phialopore—the young colony places its flagella externally. Asexual reproduction, starting from a single biflagellate cell, is essentially identical with development of the zoospore.

In both the coelenterates and the volvocine phytomonads monosexual and hermaphroditic forms are present. This and the presence of a coeloblastula-like structure only vaguely resembling the coenobium, are the only developmental similarities of these forms. The male gametes are morphologically very unlike. The volvocine zygote wall is absent in the coelenterates as is the subsequent reduction division and biflagellate zoospores. Each zoospore gives rise to a daughter colony, whereas the coelenterate zygote produces only one daughter organism. The cleavage pattern of the more complex volvocines is not seen in coelenterate development. The internally directed flagella and subsequent inversion are absent in the coelenterates. To find in the phytomonad coenobia examined here, a developmental pattern that approaches that of the coelenterates, one must exclude more highly evolved Volvocales and turn to a *Pandorina*-like organization and assume the following: (*a*) evolution of the metazoan sperm cell, with its complex posterior tail, from the anteriorly biflagellated volvocine male gamete, (*b*) loss of certain volvocine zygote characters such as the secreted wall and postzygotic reduction divisions, (*c*) loss of the motile zoospore stage (*Pandorina* typically produces a single zoospore, the other products of meiosis de-

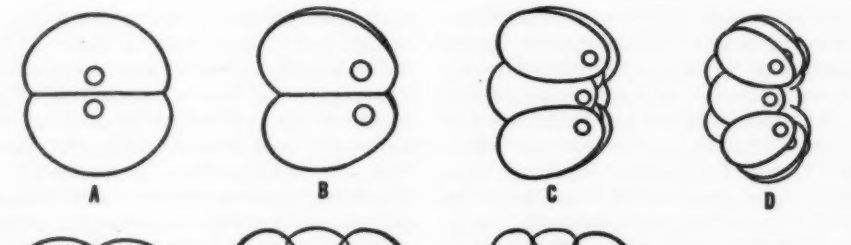


FIG. 10. Cleavage pattern in *Pleodorina illinoiensis*. *A–D*, side view of cleavage; *E–H*, same as *A–D*, seen from above. (Original by Kofoid and Merton, from Brown *The Plant Kingdom*, Ginn and Co. 1935)

generate) and (*d*) loss of the precise cleavage pattern typical of the Volvocales.

The Acoela are monoecius. Sperm are exchanged during the sexual pairing of two hermaphroditic individuals. Fertilization is, therefore, reciprocal, and is always internal. The copulatory apparatus has evolved within the group, since simpler forms possess only a male gonopore and more complex Acoela have complex copulatory structures such as a penis that transfers sperm by hypodermic impregnation. There are no definite gonads. The gametogonia lie in the central mesenchyme. Reduction divisions occur during gametogenesis. The sperm vary somewhat in morphology. Usually they are furnished with flagella-like structures at one or both ends, and often have delicate undulating membranes along each side. The eggs contain yolk, and after fertilization are encapsulated when released via the mouth or rupturing of the body. The zygotes develop into mature worms in a few days. Cleavage is spiral, determinate and unequal. The blastula is not hollow and goes directly into the stereogastrula which is the adult form. Asexual reproduction and cyst formation, both common in the other Turbellaria, are not reported for the Acoela. Somewhat limited regeneration powers are present: apparently structures such as the statocyst cannot be regenerated.

The ciliates, with the exception of the Peritrichida, are hermaphroditic. Conjugation results in cross-fertilization which is always internal. Both haploid gamete nuclei arise from divisions of the same micronucleus which lies in the inner endoplasm. The paroral cone can be considered a copulatory structure. As seen in *Paramecium* it is a protoplasmic protrusion, in which the male gamete nuclei are lodged. The male pronucleus leaves this cone which is situated posterior to the mouth, and enters the body of the other conjugant where it fuses with the female nucleus. The synkaryon undergoes mitosis. Apparently depending on their position in the cytoplasm certain of these mitotic products become micronuclei and other macronuclei (Maupas, 1889; Sonneborn, 1953). A similar situation is reported for *Tetrahymena* (Nanney, 1953). In general, conjugants dedifferentiate certain organelles, notably the gullet, which redifferentiate after completion of conjugation (Hertwig, 1889; Poljansky, 1934; Roque, 1956*a*, 1956*b*; Porter, 1956). Asexual re-

production results from transverse fission. Cysts are common. The ciliates are capable of extensive regeneration.

Two points are of especial comparative interest here. First, close similarities exist between the ciliates and acoels relative to hermaphroditism, sexual pairing, and fertilization. Second, there is a sharp difference in the gametic material and in the development of the zygote nucleus and associated cytoplasm. In the Acoela the gametes are completely cellularized, the sperm are many and self-motile, the eggs carry significant amounts of cytoplasm and yolk. In the ciliates, as a rule, the gametic elements are a single migratory (mode of movement unknown), and a single stationary haploid nucleus. The acoel zygote detaches from the protoplasm in which it originated and undergoes an independent development. In the ciliate, though the zygote nucleus lies in a somewhat undifferentiated cytoplasm, this cytoplasm has the same parameter as the whole organism. But this second difference, i.e., acoel embryogenesis as opposed to ciliate redifferentiation, depends actually on gametic cellularization, especially of the female gamete, since it is cellularization which permits detaching of the zygote from the parent organism. In the ciliates there is one exceptional group that proves gametic cellularization is not restricted to multicellular forms. In the Cycloposthidae, ciliates of the horse caecum and colon, self-motile male gametes occur (Fig. 11). These, as described by Dogiel (1925), possess a nucleated head with perforatorium and propel themselves by their tail-like structures from their site of origin in one conjugant, across the fluid-filled cavity formed by apposition of the oral structures of the conjugants, into the other conjugant where fertilization occurs. Here the male gamete is clearly cellularized. The female gamete is not and hence the probability of a detachable zygote is precluded. The importance of Dogiel's findings for this paper is that the cellularized, flagellated metazoan sperm has its counterpart in the ciliates as well as the flagellates.

In summary, then, to derive the developmental biology of the Acoela from that of the Ciliophora the major step is the cellularization of the gametes. For once this step is reached the further steps of attaining a zygote that is independent of the parent organism and which can now undergo the embryogenesis so typical of metazoan biology, is realizable. One further point is of interest here. Since the adult acoel is somatically acellular, what, if any, is the significance of cleavage of the acoel zygote? Hyman (1942) has argued that cleavage of the metazoan zygote could be either a recapitulation of colonial integration or simply the necessary part of normal development. If the Acoela are in fact evolved from ciliate-like

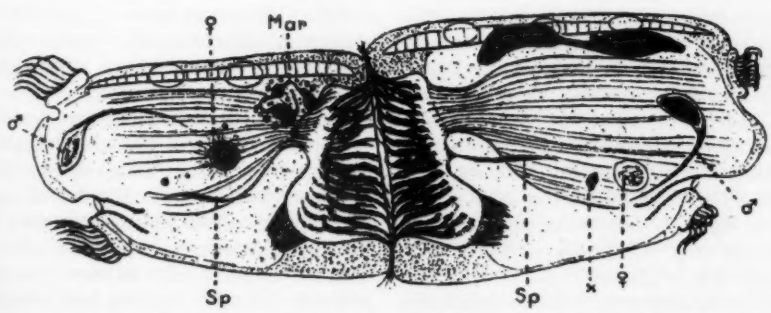


FIG. 11. Conjugation in *Cycloposthium bipolmatum*. ♂, male gamete; ♀, female gamete nucleus; *Mar*, disintegrating macronucleus; *x*, disintegrating micronucleus; *Sp*, disintegrating spindle. (From Dogiel, 1925)

ancestors, the second possibility is probably the correct one.

Comparative biochemistry and physiology. The coelenterates are carnivorous forms with both intra- and extracellular digestion. Proteolytic enzymes are reported. Locomotion is by cilia in the planula.

The colonial Volvocales photosynthesize starch utilizing chlorophyll. Colorless colonial forms are not reported. Particulate food is apparently never utilized. Locomotion is by flagella.

Information on enzyme systems and related aspects of comparative biochemistry and physiology are lacking in these forms. But, nutritionally, it is clear that the volvocine cells are photoautotrophic as compared to the chemoheterotrophic coelenterates. This difference is emphasized by the lack of colorless colonial forms, though such exist, e.g., *Polytoma,* in the unicellular forms.

The Acoela are both herbivorous and carnivorous; diatoms, algae, and copepods being found in their food vacuoles. Food is ingested via the mouth. Digestion is exclusively intracellular. There are no excretory or osmoregulatory organs. Locomotion is by cilia.

The ciliates feed on bacteria, algae, diatoms, on other protozoa, and, in the larger forms, on microscopic Metazoa. Food passes through the gullet directly to food vacuoles. Digestion is intracellular. Osmoregulatory contractile vacuoles are present in free-swimming forms and also in the marine forms with but few exceptions. Studies, especially those on *Tetrahymena* and *Paramecium,* reveal typical animal-like nutritional needs, namely, amino acids, vitamins, growth factors, and inorganic salts. Locomotion is by cilia.

In brief, nutritionally the Volvocales are plant-like, the coelenterates, ciliates and Acoels are animal-like. Hadži has suggested the origin of the Turbellarian protonephridia from the ciliate contractile vacuole, but this appears doubtful since the Acoela possess neither contractile vacuoles nor excretory organs. The enigmatic vacuoles and channels of the Acoela (see Fig. 5) which are located just below the epidermoid layer, may be important for this problem.

Ecology. The Coelenterata are predominantly marine forms. The planulae are typically free-swimming. They are abundant in shore waters; some forms are found at great depths in the ocean.

The volvocine phytomonads are all fresh-water forms. Their need for light for photosynthesis precludes survival at any great depth.

Hence, in comparing the Volvocales to the planula we must assume one of two possibilities. (*a*) The coelenterates arose in fresh water from the fresh-water volvocine ancestor, or (*b*) the ancestral volvocine was marine and its present-day survivors are the fresh-water coenobial forms and the more highly evolved marine and fresh-water coelenterates. The fresh-water origin of the coelenterates is improbable and hence the first possibility is unlikely. Regarding the second possibility all that can be said is that phytomonads are in general more common in fresh water than salt, indicating, perhaps the unlikelihood of the marine origin of the volvocines.

The Acoela are exclusively marine forms, being mostly bottom crawlers which live under stones or in the sand or mud of the littoral zone. Some are found in deeper waters (250 meters). Some are pelagic and a few species are commensals.

The Ciliophora have a great variety of habitats. Marine and fresh-water free-swimming, commensal, symbiotic, and parasitic forms are known.

For comparative purposes it suffices to note that marine, littoral, benthic, and pelagic free-living ciliates exist with habitats coextensive with the Acoela.

Ethology. Both the planula and the volvocine coenobium are positively phototropic. Beyond this meager bit no significant comparisons can be made.

The Acoela have an anterior-posterior polarity. Behavioral studies have been limited to the genus *Convoluta.* A geo-

tactic response has been described, negative when the animals are undisturbed and positive when disturbed. In a centrifugal field these flatworms tend to move against the centrifugal force. Also a positive phototaxis in white light and somewhat varying responses in variously colored lights are known. *Convoluta* is highly cathodic. It is of interest that detailed studies of the rhabdocoel *Stenostomum* have shown that the ciliary beat with reference to anode and cathode is like that in the Ciliophora. Detailed mating behavior is known only for *Amphiscolops langerhansi.* Hyman reports "a brief 'courtship' consisting of head or body contact." This is presumably similar to the "Liebesspiel" of *Convoluta* mentioned by Bresslau. Copulants press their genital regions together while facing more or less in opposite directions. This posture is common throughout the Acoela but in some forms the copulants lie parallel to each other.

In the ciliates there is anterior-posterior polarization of the body. Extensive behavioral studies have been made (see esp. Jennings, 1923). The Ciliophora are responsive to heat, chemicals, and physical contacts and usually indifferent to light except for sudden changes in light intensity. A positive rheotaxis has been reported for some forms. Positive and indifferent geotactic responses are known. Movement opposite to the direction of force in a centrifugal field is also known. In all ciliates there is the same pattern of action of the electric current on the cilia: the cilia in the anode region beat backwards, as in normal forward locomotion, while the cilia in the cathodic region are reversed, striking forward. Then, depending on the distribution of the body ciliation, different ciliates move either to the cathode, or to the anode, or remain fixed between the poles (Jennings, 1923). The mating reaction depends, at least in the holotrichous ciliates thus far studied, on the physical contact of reactive animals of complementary mating types. Only in *Euplotes,* a spirotrich, has there been found diffusible substances that can induce conjugation between members of the same mating type. In general, after the initial ciliary contact is made there is a period of many minutes duration during which the animals move freely around each other, but always in contact until, finally, the orientation suitable for conjugation is achieved. This position almost invariably involves apposition of the oral parts. Depending on the location of these parts the conjugants lie parallel, in a narrow or wide V, or even in a straight line with anterior ends (mouths) together.

General conclusions. The foregoing paragraphs can be summarized as follows, see also Table I. There is closer similarity in external morphology between the Acoela and the bilateral ciliates than between the volvocine colony and the planula. A remarkable number of features in the internal morphology of the Acoela find their parallel in the Ciliophora. The same is not true for the volvocine colony and the coelenterate blastula and stereogastrula; these forms only remotely resemble one another. Concerning cytology more information is sorely needed. Present information is that chromosome cycles are alike in ciliates and acoels, and unlike in the volvocine forms and the planula. Certain differences are present in the mitotic figures of all groups. These differences are more prominent, perhaps, in the Ciliophora and Acoela. Developmentally there is little similarity in the actual ontogenies of any of the forms discussed here. Possibilities of resolving these differences are mentioned. Distinct similarities are found in the mating phenomena of the acoels and ciliates. These are not so sharp in the volvocines and coelenterates. There are meagre data concerning their biochemistry, physiology, ethology, and ecology. All three categories agree in emphasizing similarities between the acoels and ciliates, and dissimilarities between the volvocines and the coelenterates.

It is clear that in general greater similarities and less striking differences exist

TABLE I—SUMMARY OF EVIDENCE FOR COLONIAL INTEGRATION AND CELLULARIZATION HYPOTHESES

	CILIATE-LIKE FORM AND ACOELA		VOLVOCINE COENOBIUM AND COELENTERATE PLANULA	
	SIMILARITIES	DISSIMILARITIES	SIMILARITIES	DISSIMILARITIES
EXTERNAL MORPHOLOGY	Size, shape, symmetry; cilia and ciliation; ventral mouth	Acoel gonopore absent from ciliates	Size, symmetry; flagella and cilia	Imprecise cellular organization of planula absent from coenobial forms
INTERNAL MORPHOLOGY	General body organization, absence of internal cell membranes; multinuclearity; peripherally located secretory and extrusible elements, silver staining and contractile fibrils; food vacuoles	Acoel gametogonia and nerve net absent from ciliates; acoel secretory structures more complex than in ciliates		Cellular differentiation of planula absent from coenobial forms
CYTOLOGY	Diploids with prezygotic reduction	Mitotic figures may be different		Coelenterate diploidy and prezygotic reduction absent from volvocine forms
DEVELOPMENT	Monoecious; exchange of male gametic material; internal fertilization	Acoel cellularized gametes, embryogenesis absent from ciliates	Monoecious and dioecious; oögamy.	Coelenterate sperm, embryogenesis different from volvocine counterparts
COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY	Holozoic chemoheterotrophs			Coelenterate holozoic chemoheterotrophy never found in colonial volvocines
ECOLOGY	Marine			Coelenterates predominantly marine forms, volvocales exclusively fresh water forms

between the ciliates and the acoels than between the volvocine coenobium and the coelenterate planula. Indeed, some of the most profound differences between the planula and the volvocine coenobium come from developmental studies, the very aspect considered by proponents of this phylogeny to provide the most satisfactory evidence in favor of it. It also seems to this author that insofar as a critique is possible, the gastraea forms proposed by Naef and Jägersten could in no wise be more successfully derived from the Volvocales than the planula. Smith (1938) expresses the view that the Volvocales have limited evolutionary possibilities and that "*Volvox*, the culminating member of the series, probably approaches the limit of evolution along this line." Hence, since the facts supporting the neo-Haeckelian theories are so few, and the assumptions needed to flesh out this bare skeleton of fact are so often improbably complex, it is herewith proposed that these theories be abandoned.

The alternate theory, however, is not without serious objections. First, there is the heterogeneity of the ciliates resulting from evolution within the group. This wide diversity renders it very easy to find just about any character one looks for. The parallels between the Acoela and the Ciliophora may be illusory, in terms of demonstrating a phylogenetic relationship, if it turns out that the acoel has its counterpart only in a fictional amalgam of ciliate characters that has no more reality than the mythical Chimera. It remains to be determined whether or not there is within the ciliates a single, relatively homogeneous group that is similar to the Acoela. Second, there is the ciliate macronucleus. This problem was evaded by using the term "ciliate-like," which meant like a ciliated protozoan exclusive of the macronucleus. But the important question remains unanswered, which is: Why is the macronucleus, one of the most important of all ciliate characters, absent in the Acoela? A satisfactory answer to this is necessary before Hadži's theory can be accepted. And third, the fact that somatic tissue of the Acoela is acellular whereas somatic cellularization is found in the higher Turbellaria points to the conclusion that body cellularization was achieved *within* the Turbellaria and not in the step from the ciliate-like form to the Acoela—that is, cellularization is *not* the key step from the protozoa to the Eumetazoa. What, then, is the key step?

To summarize, it is clear that neither theory as originally stated is an acceptable explanation of the origin of the Eumetazoa. The neo-Haeckelian theories of colonial integration are rejected because the volvocine coenobium and the coelenterate planula are biologically too dissimilar to be related through any hypothetical forms. The theory of origin of the Acoela from a ciliate-like form will find little support unless the three problems outlined above can be resolved.

We turn now to answering these three questions.

The pleurostomatous gymnostomes. The ciliate group most like the Acoela would be a group containing uniformly ciliated forms which are fairly large, bilaterally symmetrical, multinucleate, carnivorous or herbivorous, with a ventral mouth, and which live in a marine habitat. The Holotrichida contain the uniformly ciliated forms, and within this group the pleurostomatous gymnostomes best fit the above expectations. The Pleurostomata, a tribe proposed by Kahl (1935) and incorporated into the Rhabdophorina by Corliss and Fauré-Fremiet (Corliss, 1956) are those Gymnostomatida with slit-like or round mouths which are devoid of special ciliature and which open directly on the ventral or lateral surface of the body. Within this group there are both fresh and salt water forms. Of the latter, we shall examine in detail the genera *Remanella* and *Dileptus*, members of the Loxididae and Trachelidae, respectively (Fig. 12). The choice of *Remanella* and *Dileptus* rests on the contention that these two groups of ciliates encompass the postulated resemblances to the Acoela. First

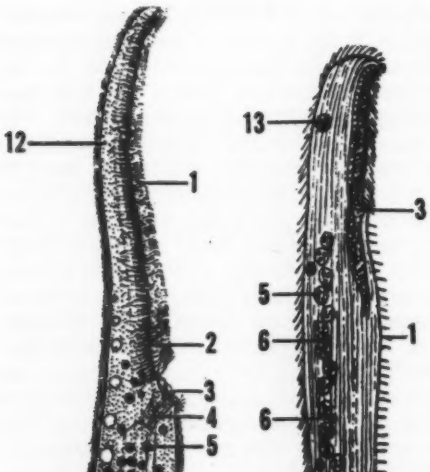


FIG. 12. *A, Dileptus anser* (From Hayes, 1938). *B, Remanella multinucleata* (From Kahl, 1935). *1,* cilium; *2,* trichocysts; *3,* mouth; *4,* trichites; *5,* macronucleus; *6,* micronucleus; *7,* food vacuole; *8,* ciliary row; *9,* rectal vesicle; *10,* tail; *11,* contractile vacuole; *12,* proboscis; *13,* Müller's vesicle.

the similarities between these Ciliophora and the Acoela will be presented, and then the dissimilarities.

Ecological similarities are these: All species of Acoela and *Remanella* are inhabitants of salt water. Most *Dileptus* are fresh-water forms, the others are marine. All *Remanella* are sandy-bottom, crawling forms, as are many of the Acoela. *Dileptus,* also, contains bottom dwelling forms, though they are not bottom crawlers. In general, the habitats of these ciliates and the acoels are quite similar, as might be expected from their food habits.

Nutritionally, *Dileptus* and *Remanella* are holozoic; the former is carnivorous, feeding on other protozoa and microscopic Metazoa, and the latter is herbivorous, feeding on diatoms and other unicellular algae. The Acoela too are holozoic.

Physiologically, no precise comparisons can be made. Only such general statements as these are possible: locomotion is by ciliary action in all cases; food is ingested via a gullet which is present in the Acoela, *Dileptus,* and *Remanella;* digestion is intracellular in all forms, the food being held in food vacuoles which lie in the inner part of the body; egestion occurs in *Dileptus* via a posterior, mid-ventral anal pore (Kahl, 1935), it is either unknown or not reported in *Remanella,* and probably occurs via the mouth in the Acoela; contractile fibrils are present in *Dileptus* and the Acoela but in neither case have they been studied physiologically. No information has been found regarding the metabolism or enzyme systems of these animals. There are no neurophysiological data. No excretory or circulatory systems or organelles are present.

The ethological information is, at best, sparse. The tropisms studied in the ciliates have not included either *Remanella* or *Dileptus* among the experimental organisms. Conjugation, reported for both ciliates (*Dileptus,* in detail by Visscher 1927; *Remanella,* one figure in Kahl, 1935), involves a homopolar alignment of the conjugants with the result that the bodies are by-and-large parallel to each other. This mating position is found in only a few Acoela. The sexual act is preceded by body (ciliary?) contacts.

The similarities between the developmental aspects of the acoels and *Remanella* and *Dileptus* are only of a very general sort. All three groups are monoecius. Also common to all three groups is exchange of male gametic material, resulting

in cross-fertilization and internal fusion of the haploid elements.

Cytologically, only generalizations are possible, but these are of a fundamental nature. There is identity of chromosome cycles and the presence of diploidy in all ciliates and Eumetazoa in general.

The internal morphology of the Acoela and gymnostomes *Dileptus* and *Remanella* shows similarities in general body organization. The remarks made earlier, concerning the Ciliophora as a whole, apply also in these specific cases in comparing ectoplasm, outer and inner endoplasm with the acoel epidermoid layer, outer and inner mesenchyme. In comparison with the multinucleate acoel body, there are certain species of multinucleate *Remanella*, e.g., *R. multinucleata* Kahl, possessing 10-14 macronuclei and 5-7 micronuclei (Fauré-Fremiet, 1954). *Dileptus anser* (Müller), an extreme case and a freshwater form, contains 100 or more small macronuclei and 20 or more small micronuclei (Hayes, 1928). The only parallel to the reproductive structures of the Acoela which is found in *Dileptus* and *Remanella* is the micronuclei. This, though a somewhat one-sided parallel, is a most important one. As regards contractile fibrils, which are common to all acoels, it is known that *Dileptus* possesses a highly active anterior trunk region. Visscher (1927*a*) reports that "Thick contractile fibrillae extend around the body in the form of a flat spiral, effecting about one complete turn for the entire length of the body." This organization of the contractile elements is different from that found in the Acoela. The only resemblance is that subsurface contractile elements are found in *Dileptus* and the Acoela. *Remanella* seems not to have been studied in this respect. The acoel statocyst perhaps has its counterpart in the Müller's vesicles of *Remanella*. This point needs more study. Finally, there are to be considered the glandular structures, rhabdoids and sagittocysts of the Acoela. Both *Dileptus* and *Remanella* possess trichocysts. In addition *Dileptus* possesses toxicysts located on the ventral side of the trunk, hence anterior to the gullet. (Visscher, 1926; Hayes, 1938). These elements are ectoplasmic. There is a similarity in these to the sagittocysts of the Acoela. The acoel frontal gland may be homologous and analogous to ciliate toxicyst-like structures. In general, in both groups the secretory elements lie close under the body surface. The Acoela, however, are more richly provided with these than the ciliates.

Lastly there are the similarities in external morphology. There is a size range overlap especially in *Dileptus* and the acoels. Larger species of *Dileptus* have individuals over one millimeter in length (Visscher 1927*a*). *Remanella multinucleata*, one of the larger *Remanella*, varies in size from 400 to 700 micra. The Acoela and *Dileptus* are bilaterally symmetrical. *Remanella* shows a modified bilaterality: designating the side with the mouth as ventral, the right body surface is uniformly ciliated, the left body surface is free of cilia and adapted to crawling over sand grains. All the forms being considered here are ciliated, the Acoela and *Dileptus* quite uniformly so, *Remanella* having the modifications just described. All forms possess mouths which lead directly into the body. The mouth is round in the Acoela and *Dileptus*, slit-like in *Remanella*.

Turning now to the dissimilarities between the Acoela and the two ciliate genera we find that as regards nutrition, physiology, ethology and ecology there appear to be no important differences. It must be emphasized, however, that in these disciplines there is a lack of rigorous studies that deal with the specific groups being examined here, and the fundamental phylogenetic significance of most of these is unclarified.

The major developmental differences are that the acoels possess cellularized gametes and these ciliates do not, and that acoels undergo embryogenesis while ciliates do not.

From a cytological viewpoint only the most general comments are possible due

to the absence of adequate studies on the Acoela. If the acoel achromatic figure is typically eumetazoan then it is centric and amphiastral, which is not true for *Dileptus* (Visscher, 1927*a;* Hayes, 1938) and in all probability not true for *Remanella.*

There are four important differences in the internal morphology of these gymnostome ciliates and the Acoela. Visscher (1927*a*) has described a "neuromotor system" in *Dileptus.* This consists of a system of fibrils composed of a rod-like motorium, just dorsal to the gullet, from which extend (*a*) fibrils to the gullet, (*b*) two thick fibrils to the proboscis, and (*c*) many fine, branching fibrils to the posterior body surface. This system is unlike the acoel nerve net in its arrangement in the animal body. We know nothing of the fine structure of the fibrils themselves, of either the *Dileptus* "neuromotor system" or of the acoel nervous system. Secondly, the acoel nuclei are spread throughout the body. In the ciliates the nuclear material is always associated with the partially fluid inner endoplasm. Thirdly, the reproductive system is vastly more complex in the Acoela than in the ciliates. And fourthly, secretory structures are more profuse and more highly developed in the Acoela than in the ciliates.

Finally, externally, the presence of a permanent male gonopore in the Acoela is a clear difference between these flatworms and *Dileptus* and *Remanella.* Certain of these worms also have a female genital pore but this is a feature that appears to have evolved within the group and therefore does not represent a primitive situation.

What now may we conclude from the foregoing?

Many of the acoel characters are indeed present in the gymnostome genera *Dileptus* and *Remanella.* However, certain important quantitative and qualitative differences also were found. Among the quantitative differences are: (1) number and distribution of diploid nuclei—more numerous and more widely distributed in the Acoela than in *Dileptus* and *Remanella;* (2) secretory elements—more numerous and more complex in the Acoela; and (3) internal and external genitalia—far more complex in the Acoela. The question now is, can the gymnostome and acoel characters of this category be considered homologous? To us, the answer, though tentative, is affirmative. Results of further comparative studies are badly needed.

Second are those differences which are apparently of a qualitative nature and whose explanations are at best highly speculative. In this category we place (1) the nerve net—present in the Acoela, absent in the gymnostomes; (2) the achromatic figure—not described but presumably centric and amphiastral in the Acoela, definitely acentric and anastral in *Dileptus* and probably also that way in *Remanella;* (3) gametic material—the acoel gametes are cellularized and the gymnostome gametes are not.

All that can be said about the nerve net is that perhaps the gymnostomes possess the potential for evolving nervous structures since, as was pointed out earlier, we see in the most complex ciliates suggestions of such an evolution. The cytological differences in the achromatic figure were discussed earlier. It was pointed out that the work of the Pollisters and of Wilson and Harvey indicate a possible resolution of this difficulty. As briefly stated earlier, the major differences in the developmental aspects arise from one key point, which is that the Acoela, like all Eumetazoa, possess cellularized male and female gametes, and *Dileptus* and *Remanella,* like most Ciliophora, do not. Cellularized germinal material results in sexual reproduction, the potentiality of new genetic combinations through fertilization together with biological increase in number.[8] And in sexual reproduction there is formation of a zygote, detachable from the

[8] Conjugation is not considered here as sexual reproduction: it is simply a sexual process. For conjugation results in no biological increase in number but does carry the possibility of new genotypes through fertilization.

parent organism, which makes possible embryogenesis, a fundamental character of the Metazoa. All this follows from cellularized gametes. The problem, therefore, comes down to the presence of noncellular gametic nuclei in these Ciliophora and of cellular gametes of the Acoela. The question then arises whether or not cellularized gametes can evolve from a situation such as that found in the gymnostomes. To this there is no good answer. We can only repeat that the presence of cellularized male gametes in certain complex ciliates (p. 29) proves that cellularization of gametic material is possible within certain Ciliophora.

However, even if we assume that the nerve net, achromatic figure, and cellularized gametes do represent clear-cut differences between the Acoela and *Dileptus* and *Remanella,* with no chance that subsequent research might mitigate the differences, it is this author's opinion that the remarkably close similarities found in so many of the other important aspects of the biology of these groups, speak more in favor of the biological resemblance of these forms than against it. Therefore, it is concluded that there is within the ciliates a relatively homogeneous group which does resemble quite closely the Acoela.

The ciliate macronucleus. If a ciliate-like form is to be considered ancestral to the Acoela, there is the problem of explaining why the macronucleus, a fundamental ciliate character, is not found in the Acoela. The problem can be approached by determining whether or not there is a set of nuclear phenomena which could represent a graded series joining the many, widely dispersed diploid nuclei of the Acoela at one extreme to the hyperpolyploid macronucleus (Fauré-Fremiet, 1953; Sonneborn, 1954) and diploid macronucleus of the Ciliophora at the other extreme.

Let us start by briefly reviewing the remarkable nuclear situation in *Remanella.* Fauré-Fremiet (1954) has presented evidence that in *Remanella multinucleata* there is no reproduction of the macronucleus *per se.* Macronuclei are continuously arising directly from micronuclei, which also produce more micronuclei. The macronuclei so formed apparently do not persist indefinitely but eventually degenerate. This situation is in sharp contrast to that typical for the ciliates in general. The common ciliate situation is that the macronucleus originally comes from the fertilization synkaryon, which is formed during conjugation. Thereafter during vegetative reproduction the macronucleus divides amitotically in synchrony with fission. (*Dileptus anser* is an interesting exception in this respect since Hayes (1938) reports that dividing macronuclei can always be found in the interfission period).

It can be seen that the nuclear situation in *Remanella* provides a transitional form in a possible series going from that typical of the ciliates to the dispersed nuclei of the acoels. The series is: (*a*) Typical ciliates with a macronucleus which reproduces itself during fission but which disintegrates during sexual processes and is reconstituted from a diploid nucleus; (*b*) *Remanella,* in which the macronuclei cannot divide and which continuously arise from the many diploid nuclei (these latter in all probability arise from the same synkaryon and are therefore isogenic); (c) the Acoela, with no macronuclei, in which many diploid nuclei are present (all of which come from the same zygote nucleus).

The hypothetical ciliate-like form would fit between (*b*) and (*c*). It would differ from (*c*), the Acoela, in that there would probably be fewer nuclei and these would be centrally located, i.e., not so widely distributed as in the flatworms. It would differ from (*b*), *Remanella,* in that it would possess no macronucleus; this would be consistent with Hadži's conception of this form. We have, therefore, no sharp gap between the ciliates and the Acoels.

This series, however, says nothing as to whether the ciliate-like form was derived

from the ciliates and in turn gave rise to the Acoela, or whether it was the progenitor of both the Ciliophora and the Acoela. Or whether, even, it evolved from the Acoela and gave rise to the ciliates. This can best be answered in the context of general phylogenetic considerations. Before going to that we must face the problem of just what the difference is between the ciliate-like form and the Acoela, namely, what might be the transitional step from the protozoa to the Eumetazoa.

The transition from ciliated protozoan to acoel. As pointed out earlier, the absence of cell membranes in the acoel soma and the presence of such membranes in the higher Turbellaria, leads to the conclusion that cellularization has evolved within the Turbellaria. The step from the Protista to the Eumetazoa is, therefore, not cellularization but something else. The following hypothetical explanation must be considered as highly speculative.

The major difference between the Acoela and their hypothetical ciliated ancestor is complexity—morphological and physiological. We propose that this stems from the placement of the nuclear material. In the acoels this material is widely dispersed. In the ciliate-like ancestor, as in ciliates in general, the nuclei lie only in the more fluid, inner, cyclotic endoplasm. What is the significance of this difference? Let us speak here in terms of energids. An energid is a physiological unit of nucleus and cytoplasm (Sachs, 1893). Cell membranes are irrelevant to this concept. Both the Acoela and the hypothetical ciliate-like form are polyenergid (as are also *Remanella* and *Dileptus*). However, and here we are approaching the crucial point, since the nuclear material of the ciliated form is probably isogenic (we assume conjugation occurs), and since it lies in the inner regions of the organism where each nuclear body is surrounded by essentially similar cytoplasm due to the constant circulation of at least part of the inner endoplasm, it might well be concluded that the energids are physiologically equivalent since each would possess the same genotype and the same intra-organismic environment. On the other hand, in the Acoela, though the genotypes of the energids are presumably identical, the intra-organismic environment would *not* be the same for all the energids. Because here the energids are scattered throughout the body in relatively fixed areas, such as epidermoid layer, outer and inner mesenchyme, and gradients of external and internal factors are probably present. Therefore, energids lying in different intra-organismic environments might well be physiologically different despite their isogenicity. Hence the crucial point: the polyenergid system of the ciliate-like form is suggested as being physiologically *compound* and that of the Acoela as being physiologically *complex*. The Acoela are therefore no longer protozoan but truly metazoan in the sense that within different parts of the same body energids possessing identical genotypes are involved in different functions. The critical point in this hypothesis is whether or not isogenic energids in different environments manifest different phenotypes. If we deal with populations of isolated energids, that is, microbial populations, the answer is, yes. Here we do not have in mind selection experiments, i.e., the random appearance of rare mutants. We refer to cases of directed changes which affect the whole experimental population of micro-organisms. In this category are induction of enzyme formation in bacteria (Davies and Gale, 1953) and certain studies on the protozoan *Paramecium,* notably those bearing on the formation of specific ciliary antigens (Sonneborn, 1948; Beale, 1954). The next question, does this also occur in the energids or cells of individual metazoans, is as yet unanswered. Certain lines of evidence show that environmental factors can affect differentiation itself and also the stability of differentiated tissue (reviewed by Trinkaus, 1956). Just how critical these factors are is still unresolved.

This postulated attainment of a com-

plex from a previously compound entity is another example of what Sonneborn has called "the fundamental tactic of evolution; repeat, then vary." Each step of this tactic starts from a basic unit whose number is increased in some manner to result in a localized compound structure. Variations occur within the units of this compound structure giving rise to a complex structure. The complex structure now becomes the organizational unit for forming a new compound structure at a higher, more complicated level of organization. This tactic extends from molecules and polymerization, to genes and chromosomes, to energids and cells and tissues, to segmentation, to populations of organisms, and to societies (Dogiel, 1929; Müller, 1947; Sonneborn, 1955). The step from the Protista to the Eumetazoa uses the energids as the organizational unit.

Is there a connection between energid complexity and cellularization? The appearance of cell membranes is here thought of as reinforcing energid heterogeneity. This compartmentalization would be of value in terms of promoting further physiological and morphological differentiation. But what is the origin of cell membranes between energids? Modern electron microscopy points to a possible answer.[9] The endoplasmic reticulum has been found in all animal forms studied. But especially important for the present discussion is that it appears probable the reticulum is continuous with the cell membrane (Pallade, 1956). If this observation is generally applicable, the origin of cell membranes seems, at least anatomically, quite straightforward. Between the energids the reticulum has become a permanent structure. The appearance of cell membranes within a single mass of protoplasm is of course common in the multiple fission that can occur in the multinucleate plasmodia of many Protista.

Finally, let us consider briefly the cellularization of the acoel gametes. We suggest that cellularization has been allometric in the Acoela, the germ line becoming cellular before the soma. This differential rate of cellularization might be explained in these terms. As pointed out earlier, cellularized gametes achieved for the first time sexual reproduction in animals, a process of great evolutionary significance (see for example, Dobzhansky, 1950). Cellularization of the acoel soma, on the other hand, perhaps had no such immediate and far reaching advantages, namely, it was of no selective importance whether or not the somatic energids were cellularized. In the other, on the whole larger, Turbellaria, somatic cellularization was, on this view, selectively important, perhaps in achieving further body complexity in the form of true tissues and organ systems.

In summary: An attempt has been made to answer the three criticisms which precluded acceptance of Hadži's proposals (see p. 33). It is concluded that in each case the criticisms can be met in such a manner as to justify the acceptance of Hadži's proposed origin of the Acoela from a ciliate-like ancestor.

General Remarks

Phylogeny. Since the gymnostomatous ciliates are considered to be the most primitive of the Ciliophora (Hyman, 1940; Corliss, 1956), it suggests that the series (*a*) Ciliophora, exclusive of certain gymnostomes, (*b*) primitive gymnostomes such as *Remanella* and *Dileptus,* (*c*) the hypothetical ciliate-like form, and (*d*) Acoela, can be represented for phylogenetic purposes as V-shaped. The hypothetical ciliated form is at the base of the V. The primitive gymnostomes and

[9] The author is indebted to Dr. Jack Schultz for pointing out this possibility. Furthermore, it should be emphasized that this mode of cellularization has nothing in common with the idea sometimes discussed (e.g., Baker, 1953) that the eumetazoan, if it arises from a single multinucleate protistan, is cellularized because of incomplete separation of fission products of the protistan. Such a view is really only another modification of the Haeckelian view.

then the rest of the ciliates complete one arm of the V. The acoels are represented by the other arm. This proposal means that the hypothetical organism is ancestral to both the Ciliophora and the Eumetazoa.

The hypothetical form, arguing from the biology of the Acoela and Gymnostomatida, can be characterized in the following terms. It is bilaterally symmetrical, uniformly ciliated, and possesses a mouth that is not apically situated. There are many diploid nuclei lying in the inner endoplasm, which is the more fluid part of the body. In this region also lie the food vacuoles. Nuclear membranes are permanent and mitosis is an orthomitosis —the centromeres attach to the mitotic spindle. The achromatic figure is probably anastral and acentric. Cytoplasmic particles typical of animal protoplasm are present. Conjugation occurs. Haploid gamete nuclei are formed, are exchanged between the conjugants, and achieve an internal fertilization which restores the diploid number to the synkaryon so formed. Asexual reproduction is by transverse or homothetogenic fission. The organism is holozoic. It is a free-swimming, benthic, marine form.

How did such a form arise? In all probability the Ciliophora arose from the zooflagellates (Hyman, 1940; Grassé, 1953; Kudo, 1954; Corliss, 1956), hence it is assumed that the hypothetical ciliate had a zooflagellate ancestor. Arguing from the proposed characteristics of this protociliate, the hypothetical ancestral flagellate is thought to possess the following features. The body is probably bilaterally symmetrical with many mastigonts, i.e., units of basal granule, one or more flagella and associated structures. There would be many nuclei, possibly diploid, and not associated with the mastigonts. (The latter are therefore akaryomastigonts.) Orthomitosis and anastral, acentric, achromatic figures are expected along with constancy of the nuclear membranes. A sexual process involving union of two separate organisms is also expected, plus fusion of haploid nuclear material. Longitudinal or symmetrigenic fission, typical of the flagellates, is in all probability present. Holozoic nutrition and a free-swimming, marine habitat complete the description.

The foregoing raises four major problems. 1. What is the plausibility of the hypothetical flagellate? 2. What is the plausibility of deriving the hypothetical protociliate from this flagellate? 3. Are there any reasons, other than the primitive nature of the gymnostomes, for proposing the evolutionary dichotomy of the acoels and ciliates from the protociliate? 4. How does all this relate to Hadži's proposals? We shall take these questions in order.

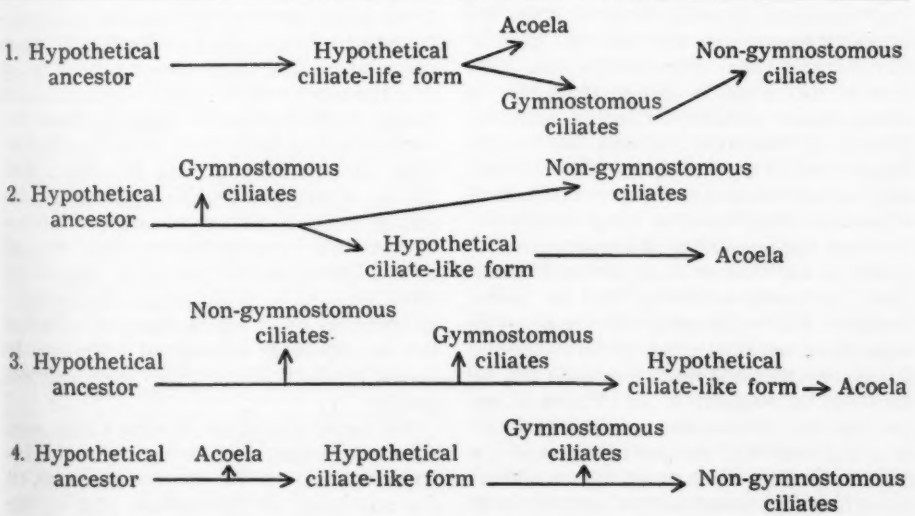
The zooflagellate genus *Calonympha* resembles the hypothetical flagellated form in several important respects. This member of the Trichomonadida possesses many akaryomastigonts, and is multinucleate. The nuclear membrane is always present, and the achromatic figure is anastral and acentric. Fission is symmetrigenic. Here the resemblances cease. However, in other members of the higher zooflagellates, i.e., the Polymastigida and the Hypermastigida, there are found some of the other characters of the hypothetical flagellate. Bilateral symmetry is found in *Giardia*. Cytostomes have been reported in certain forms, but there still seems to be doubt as to whether they function as gullets (Grassé, 1952). In many Hypermastigida there is orthomitosis (in *Calonympha* karyokinesis is pleuromitotic with centromeres apparently attached to the nuclear membrane). Cleveland (1956) has reported sexual fusion in the Hypermastigida. Also, he reports certain members of this order are diploid, namely, *Urinympha* and *Notila*, and show a prezygotic meiosis. However, none of the forms mentioned thus far is free-swimming. All are parasitic, endocommensals, or symbiotic. Its mode of life is, therefore, the only characteristic postulated for the hypothetical flagellate that is not found in the zooflagellates mentioned above. And

since, in any case, it is always dangerous to argue about free-living forms from non-free living ones, it is concluded that the hypothetical flagellate is not a very plausible form.

The following is suggested to explain the absence of free-swimming, highly differentiated flagellates, and, if it is indeed a reasonable suggestion, it may alleviate somewhat the implausibility of the hypothetical flagellate. Let us assume there were free-swimming marine zooflagellates that possessed many flagella and nuclei. (Certain present-day trichomonads are marine and free-swimming.) If our phylogeny is correct these hypothetical zooflagellates eventually evolved the ciliates and acoels. These latter two groups, perhaps more highly adapted, might have selectively eliminated the flagellates. The only flagellates to survive this competition would be those in highly specialized habitats. Perhaps the fact that today the more complex zooflagellates are predominantly parasitic is the result of this adaptation to quite specific niches. Present-day zooflagellates would thus represent, in a sense, relict populations. The domains where their forebears were once free-swimming have been pre-empted by the ciliates and microscopic Metazoa.

The second question raised above challenges the origin of the ciliated forms from the zooflagellates. Little evidence supports this proposal, especially since likely candidates at the zooflagellate end of this phylogeny are hard to come by, as just pointed out. But we can picture flagella shortening to cilia, as in the flagellate *Opalina* (Grassé, 1952; Corliss, 1955). Perhaps complete sexual fusion was inhibited and this led to conjugation. And, in some way symmetrigenic fission was replaced by homothetogenic fission. These speculations, plus the scattered resemblances to the protociliate, e.g., bilaterality, gullets, multinuclearity, diploidy, orthomitosis, and prezygotic reduction are representative of the data that might be thought to argue for the zooflagellate ancestry of the ciliates.

The third question asks for the evidence favoring the postulated V-shaped evolution. *A priori,* in addition to this V-shaped evolutionary pathway, there are three other possibilities which preserve the sequence of forms which has the Acoela and the non-gymnostomous ciliates as the two terminal groups.



The third possibility is highly improbable in that there is no good evidence which suggests that the non-gymnostomes were evolved before the gymnostome ciliates. For this reason we consider this possibility the least probable of the four.

Of the remaining three schemes the second one seems least likely simply on the basis of parsimony. In this case it must be postulated that a macronucleus was evolved and then lost again. This is not necessary for the first and fourth possibilities.

In evaluating the fourth possibility two problems are apparent. First, what is the origin of the Acoela? Second, is it useful to think of the ciliates as derived from acoels? To answer the first question there is the planuloid-acoeloid theory (see Hyman, 1950) according to which the Acoela evolved from a planula-like ancestor, in other words, from other eumetazoans. Later we will return to this theory in another connection. At present, this theory, if accepted, achieves the isolation of the Eumetazoa and the Ciliophora from the Protista. For there now remains no plausible theory for the origin of the planula—the flagellates are not plausible ancestors, as we showed earlier; nor are the ciliates candidates—though more similar to the planula in many respects than the flagellates—because the present speculation derives them from the Acoela. This line of thinking is found therefore to create more problems than it answers. Though it does not disprove the theory, one is lead to cast about for alternatives. Such an alternative is the first suggestion, which we shall turn to after briefly examining the possibility of degenerate evolution of the ciliates from acoeloid ancestors. Although realizing that no protozoologist has apparently ever taken this suggestion seriously, let us nevertheless do so now and see what evidence can be gathered in support of it? There is the fact that the ciliates and acoels are similar in many essential respects. Perhaps the outstanding problem is to try to understand how the macronucleus evolved from the Acoela. If this structure is a token of degenerate evolution, then what is the explanation of the macronucleus in certain Foraminifera, e.g., *Iridia lucida* and *Rotaliella heterocaryotica* (Grell, 1954, Le Calvez, 1952)? From what have these foraminifers degenerated? There seems to be no reasonable answer. We are again left with more problems than solutions. We must again cast about for alternatives.

In the first possibility, the major problem is the plausibility of the hypothetical protociliate's having evolved both dispersed nuclei and endopolyploidy. The existence of these nuclear conditions suggests that a problem of nucleocytoplasmic ratio may have existed in the protociliate which was solved either by many small nuclei or by a few large nuclei. A mutant in which the wide dispersion of nuclei became permanent could be the progenitor of the Acoela. Such a dispersion might occur during a sexual process for we know that during this process in the ciliates extensive protoplasmic reorganization is in progress (e.g., in *Paramecium* see Maupas, 1889; Hertwig, 1889; Diller, 1936; Sonneborn, 1953; Roque 1956*a*, 1956*b*; Porter, 1956). Where the nuclei were not dispersed endopolyploidy will be a possible answer. In this case appropriate mutations might then transform the polymacronucleate *Remanella*-like form, with macronuclei unable to divide or persist long, into the more efficient monomacronucleate forms, typical of most ciliates. Here the macronucleus, largely autonomous, can divide and, in some forms, persist indefinitely. Furthermore, the suggestion that endopolyploidy is an answer to nucleocytoplasmic ratio problems, also applies to the Foraminifera. In *Iridia lucida* the large amount of cytoplasm in the gamont is serviced by a macronucleus, whereas the equally large amount of cytoplasm is, in the schizont, handled by many diploid nuclei!

We come, therefore, to this conclusion. An evolutionary dichotomy from the hypothetical ciliate-like form to give rise, on the one hand, to the ciliates and, on the

other, to the acoels, seems to be the most satisfactory overall explanation. Other explanations are not excluded, but they necessitate far more complicated suppositions. Hence we must admit that the choice of solutions here really stems from an application of Occam's razor.[10]

Finally, there is the question of relating all the foregoing to Hadži's phylogenetic proposals in this field. This is quite simple and obvious. The hypothetical protociliate is the equivalent of Hadži's "Infusoria-like" form. But Hadži's eminently plausible phylogeny should not be referred to, as was done earlier, by the phrase "cellularization of the individual." If the views expressed in this paper should prove to be correct concerning the fundamental transitional step from the Protozoa to the Eumetazoa, a more appropriate phrase might be *differentiation of energid heterogeneity*. At this point we insert our answer to Metschnikoff's criticism of "the cellularization hypothesis" (see p. 18). The biogenetic law no longer has the paramount import it had in the latter half of the nineteenth century (de Beer, 1940; Hyman, 1940), hence we do not feel constrained to argue with Metschnikoff that acceptance of the theory of differentiation of energid heterogeneity demands, also, acceptance of the pterygote insects as the most primitive eumetazoans. Furthermore, it is well to be clear on another somewhat related point. Acceptance of Hadži's theory means that acoel embryogenesis recapitulates nothing since this is the first time, in the evolutionary sense, that embryos have appeared. To put it another way, if we were asked, "which came first, the acoel or its egg?" Our answer would be the acoel.

The phylogeny proposed here is presented in the upper right-hand portion of Figure 13. The rest of this figure is discussed in the section that follows.

Multicellular forms and cellularity. The phylogenetic relationship of the rest of the Eumetazoa and especially the Coelenterata to the Acoela needs mention. Hadži (1953 and earlier works) has proposed the origin of the coelenterates from turbellarian rhabdocoels by regressive evolution. The ecology and feeding habits of the coelenterates, it is argued, have resulted in the replacement of an initial bilateral symmetry by the present day radial symmetry. In Hadži's words, ". . . Eumetazoa which possess a primary radial symmetry do not exist." Hyman (1951) defends the planuloid-acoeloid theory of origin of the Bilateria as the best theory of the origin of bilateral Metazoa. There appears no reason why the arguments of this theory cannot be reversed to account for the origin of the planula from the Acoela with the following modification. Whereas the Acoela already possess a mouth, the mouthless planula would be derived from the mouthless ciliated, cellularized, larval form of the acoels. It is beyond the purpose of this paper to evaluate the comparative merits of the origin of the coelenterates from the Acoela or the Rhabdocoela. The point to be made is that the Coelenterata seem clearly to be derivable from the Turbellaria, as are the rest of the Eumetazoa. Thus the Turbellaria, more specifically, the primitive acoeloid forms, represent the starting point of eumetazoan evolutionary radiation.

The sponges are universally considered as an evolutionary dead end. But they are without doubt animal-like and multi-

[10] Another piece of work is highly suggestive for the present problem. F. R. Lillie (1902, 1906) using the annelid *Chaetopterus* induced the development of an acellular trochopore by exposure to a KCl solution. The point of especial interest here is that in this abnormal, non-cellular larva, originating from a single fertilization nucleus, the nuclear material was organized in two ways: either as many small nuclei scattered in the inner portion of the larva, or, as one large polyploid nucleus. The parallel is remarkable to the developmental dichotomy being proposed here for the origin of the ciliates and acoels from the protociliate. We are reminded of Goldschmidt's (1952) comment that ". . . evolution [is] not only a statistical genetic problem but also one of the developmental potentialities of the organism."

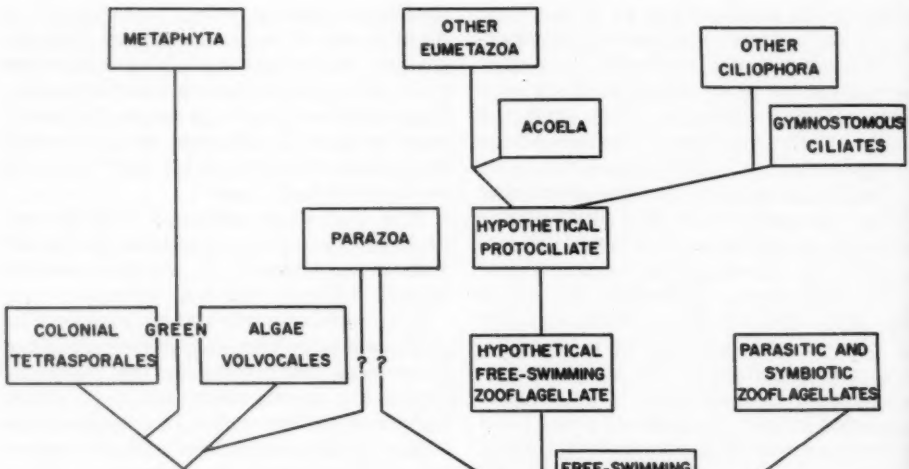


FIG. 13. Summary of proposed phylogeny.

cellular. A widely accepted theory of their origin is they evolved from protistan collared flagellates. The presence of choanocytes supports this theory. However, the embryology of certain sponge groups is reminiscent of volvocine development (Hyman, 1942). In any case integration of a flagellate colony is clearly indicated. From this the argument is sometimes voiced that since these Metazoa most probably arose by colonial integration why not the same for the Eumetazoa? But this argument is two-edged, as pointed out by de Beer (1954). The Porifera are, as unanimously conceded, still at the cellular grade of construction—only highly developed colonies. Therefore, why expect any other evolutionary experiment of this sort to be more successful than the sponges? It seems to us that the sponges are clearly of a different origin than the Eumetazoa and probably represent the limit of evolution in animal-like forms which utilize the principle of colonial integration. This principle has met quite another degree of success in plant-like forms.

There are so many examples of closely related unicellular and multicellular algae, and their transitional forms, that the evolution of the multicellular state through aggregation of unicellular forms is scarcely to be doubted in the Thallophyta. The Metaphyta in all probability arose from the tetrasporalian colonial green algae. Hence, if the theory of the origin of the Eumetazoa proposed here, i.e., formation of a physiologically complex

polyenergid form from a physiologically compound polyenergid form followed by cellularization, is correct, then the Eumetazoa stand alone among multicellular forms in their method of achieving the multicellular state. This is perhaps most clearly brought out by stating one of its necessary conclusions. The metazoan *organism* is homologous to one of the constituent cells of a multicellular plant.

Furthermore, perhaps it is not surprising that among the Eumetazoa we have the most complex biological creatures. For their history, according to the view discussed here, has occurred without break *within* an individual organism. Nowhere has the historical continuity of a single coordinated protoplasmic mass been broken, as by colony formation and subsequent integration. It would seem that evolution within a differentiated polyenergid individual is capable of greater physiological and morphological complexity than evolution in which a mass of individual cells is integrated; the evolutionary potential of the delicately coordinated and intimate *intra*-organismic interrelations cannot be recovered at the *inter*-organismic level.

Summary

Integration of a protistan colony or cellularization of a protistan individual are the two theoretical modes of evolving the cellular or multicellular state from the acellular or unicellular one. Differing series of biological forms have been proposed to accord with one or the other of these hypothetical principles. The neo-Haeckelian proposals, utilizing the principle of colonial integration, all start from volvocine colonies in attempts to evolve variously the coelenterate stereogastrula, or the hypothetical metagastraea or bilaterogastraea. The two hypothetical forms are not sufficiently well characterized biologically to be of use in a rigorous critique. In the case of the planula and volvocine colony it was concluded that these forms are so biologically dissimilar that the chance of evolution along this line is highly improbable. Utilizing the principle of cellularization of an individual Hadži has proposed that a polyenergid ciliate-like form evolved the primitive free-swimming, turbellarian Acoela. This is held to be a plausible theory providing three major criticisms can be met. These are: (1) Is there a group within the ciliates which sufficiently resembles the Acoela as to render reasonable a hypothetical ciliate-like form which bridges the ciliates and acoels in this theory? (2) Can the presence of the macronucleus in the Ciliophora and its absence in the Acoela be explained? (3) Since cellularization cannot be the major transitional step from the Protista to the Eumetazoa—both the Ciliophora and the somatic structures of the Acoela are acellular—what is the nature of this step? The answers proposed for these three questions are: (1) Certain pleurostomatous gymnostomes, members of the holotrichous ciliates, are considered to be ciliated protozoa which closely resemble the Acoela. (2) The problem of the macronucleus is answered by presenting a graded transition from (*a*) forms with well-developed macronuclei, to (*b*) a pleurostomatous gymnostome, i.e., *Remanella*, with what is interpreted as a primitive macronuclear condition, to (*c*) the hypothetical ciliate which is postulated to be multinucleate but lacking macronuclear structures, to (*d*) the Acoela. (3) It is suggested that a multinucleate ciliated form with nucleo-cytoplasmic units of essentially similar physiological activities gave rise to a form in which these units possessed different activities, i.e., a physiologically compound polyenergid form gave rise to a physiologically complex polyenergid form. Cellularization followed upon this step. These considerations lead to the preference of Hadži's proposed origin of the Acoela from an Infusoria-like ancestor.

A phylogeny for the origin of the Eumetazoa is proposed which starts with a hypothetical multinucleate, polyakaryomastigont flagellate. This gives rise to the protociliate, which is Hadži's ciliate-like

form. From this the Acoela and Ciliophora proper were evolved as different evolutionary lines. The problem of the origin of multicellular forms in general is briefly discussed.

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Points of View

Taxonomic Characters and Infraspecific Variation in Mollusks

In the past several years a number of excellent discussions have been presented in SYSTEMATIC ZOOLOGY concerning the treatment of the lower taxonomic categories. In view of this it is difficult to understand the recent suggestions presented by John Q. Burch (1956) regarding taxonomic characters in mollusks. Although we agree that extreme splitting has made mollusk systematics confusing in a number of cases, his proposals would enhance the tendency for molluscan systematic structure to become "too nebulous for any to comprehend." The delineation of all the categories considered by Burch are inadequate, but the statement "*subspecies* should be based on characters resulting from environment" causes us most concern. This connotes (1) that infraspecific variation results as a direct effect of the environment on the genetic system—a Lamarckian viewpoint not currently favored by most biologists; (2) that the variations result indirectly through natural selection of genotypes most compatible to the environments; or, (3) that variations result by expression of the gene complex in different ways in different environments. The first suggestion will not be dealt with here. That the second occurs is a major thesis of Darwinian evolution. That the third commonly occurs in mollusks (the "ecotypes" and "ecophenotypes") is well known to malacologists.

Many mollusks tend to form a great number of isolated local populations because of their sedentary habits and wide distribution. Often these populations show a marked homogeneity among members of each population and often nearly every local population may be morphologically distinguished from all others. These "microgeographical races" may be either genetically determined or ecophenotypically conditioned. To separate the ecophenotype as a taxonomic unit with a subspecific name is erroneous in principle, as has been pointed out by Hubendick (1951, p. 34). If the morphological characters of the populations are genetically determined, recognizing each population as a subspecies (or species) because there exists a clear-cut discontinuity of characters is absurd. The list of latinized names for many species would conceivably number in the thousands, confusing even the most ardent students of molluscan taxonomy. The taxonomic treatment of such genetically distinct microgeographical races in snails has been discussed by Wilson and Brown (1953, pp. 102-103). A method by which ecophenotypes might be handled has been presented by Edwards (1954).

We feel that there is more to systematics than merely "the identification of the animals involved," but we agree with Burch that it is "logical not to make the system unnecessarily complex." It is essential that we try to recognize and understand the underlying mechanisms and principles of variation, speciation, and evolution, realize the inadequacy of our system of nomenclature in respect to dynamic biology, and attempt to classify our animals accordingly.

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Contributions of the following types are solicited: Papers on principles and the applications of principles of wide implication and general interest in any phase of systematics, such as comparative anatomy, zoogeography, paleontology, taxonomy, classification, evolution, or genetics; discussions of methods, specific problems, and activities of systematists; discussions of new books and monographs; and news of systematists, organizations interested in systematics, research and teaching programs, expeditions, collections, meetings, and anything else of interest to systematists.

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